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THESE DE DOCTORAT DE

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Sciences de la Mer et du littoral
Spécialité : *Ecologie marine*

Par

Youenn JÉZÉQUEL

**« Ecologie acoustique du homard Européen (*Homarus gammarus*)
et de la langouste rouge (*Palinurus elephas*) »**

Thèse présentée et soutenue à Plouzané, le 06/10/2020
Unité de recherche : Laboratoire des Sciences de l'Environnement Marin (UMR 6539)

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« La raison pour laquelle j'aime la mer, je ne peux pas l'expliquer - c'est physique. Lorsque vous plongez, vous commencez à vous sentir comme un ange. C'est une libération de votre poids. »

Jacques-Yves Cousteau.

Remerciements

J'ai eu la chance d'être attiré par le milieu marin depuis tout petit par le biais de la pêche, avec cette fascination (presque malade) pour le homard (même si maintenant je préfère la langouste). A la sortie du lycée, j'étais complètement perdu dans mon choix d'orientation. Pourtant, suite à de surprenantes rencontres mêlées à une bonne dose de hasard (et/ou de chance), je viens tout juste de terminer une thèse en écologie marine dont le but était d'étudier mon animal préféré. J'ai ainsi eu la chance, durant ces trois dernières années, de combiner travail et passion, mais aussi de voyager (plusieurs fois) hors de ma Bretagne (ce qui n'était pas gagné au départ) et de rencontrer des personnes exceptionnelles. Cette section du manuscrit aura été de loin la plus difficile à écrire, tant je tenais à remercier toutes les personnes qui ont participé, de près comme de loin, à cette incroyable aventure.

Je souhaite tout d'abord remercier l'Université de Bretagne Occidentale pour avoir financé entièrement cette thèse. Je remercie en particulier Catherine Meur Ferec (directrice de l'école doctorale) qui avait jugé favorablement mon oral de candidature de thèse, ce qui m'a permis d'obtenir cette bourse.

Je tiens ensuite à remercier Olivier Adam, Christopher McKindsey et Isabelle Charrier pour avoir accepté d'être rapporteurs et rapportrice de ce manuscrit. Merci également à Stéphanie Thiébault et Jérôme Sueur d'avoir évalué ce travail en tant qu'examinatrice et examinateur, et Anne Lebourges en tant qu'invitée. Vos commentaires et remarques ont grandement amélioré ma vision des perspectives issues de cette thèse.

J'en viens maintenant à remercier la seule personne en France (voir dans le monde ?) capable de proposer des sujets de recherche aussi surprenant que la bioacoustique du homard. L'histoire commence lorsque j'ai rencontré Laurent Chauvaud pour discuter des variations saisonnières du recrutement de la crépidule en rade de Brest lors de mon stage de première année de Master à l'IUEM. Pour le remercier, je lui avais donné quelques homards fraîchement pêchés, et je m'en souviens encore mot pour mot, qu'il m'avait dit « Tu as l'air d'aimer cette bestiole, on développe la bioacoustique au labo, je suis sûr que ces homards émettent des sons, si tu veux je te prends en stage de M2 sur ce sujet, et si ça marche on tente d'en faire une thèse ». Et comme toute idée farfelue lancée par Laurent Chauvaud au détour d'un couloir, forcément ça a marché... Encore un grand merci pour m'avoir donné ta confiance en me laissant le champs libre pour faire ce que je voulais pendant cette thèse, je réalise bien la chance que j'ai eu de t'avoir comme directeur de thèse. Merci pour ton soutien, surtout pendant cette période difficile de la 2^{ème} année, et pour toute ton aide et le matos que j'ai pu utiliser pendant les manips. Merci aussi pour tous ces moments passés à discuter autour d'un barbecue homard (ou langouste, pour changer un peu), et pour toutes les soirées passées chez toi. Il n'y a qu'une seule chose que je regrette, c'est de ne pas avoir plongé avec toi durant nos manips *in situ*, j'espère qu'on se rattrapera plus tard, peut-être en Antarctique?

Dans le mot bioacoustique, il y a le terme acoustique (qui prends d'ailleurs plus de place que le bio de biologie, étymologiquement parlant), c'est-à-dire un mélange entre les sciences de l'ingénieur (l'étude des sons) et le traitement du signal, avec des équations mathématiques qui font peur à tant de biologistes. J'ai eu la chance d'avoir comme directeur de thèse un des dieux de l'acoustique mondiale, Julien Bonnel. Enseigner les bases du traitement du signal et du codage sur matlab n'est pas simple face à un biologiste homardologue, et je souhaite te remercier pour ta grande pédagogie et ta patience. Même si un océan nous séparait pendant ces trois années, tu étais toujours réactif pour répondre à mes (nombreux) messages, ou corriger des articles dans un temps record (!). Merci aussi pour la qualité scientifique que tu as su me transmettre, que ce soit dans le développement des protocoles, des manips, de l'analyse des résultats, et aussi pour les réponses aux reviewers. Un énorme merci pour m'avoir permis de quitter ma Bretagne pour aller au Canada et aux US pour participer à deux conférences de l'Acoustical Society of America à Victoria (2018) et San Diego (2019). Merci aussi pour ces deux séjours inoubliables passés à Woods Hole (2019 et 2020), même si le deuxième aura été écourté à cause de la crise Covid. Ça a été vraiment un grand plaisir de travailler avec toi pendant ces trois ans (et demi), et j'espère que ce n'est qu'un début (il y a encore tellement de choses à découvrir).

Merci également à Jennifer Coston-Guarini pour avoir encadrée une bonne partie de cette thèse. Au début, j'étais un étudiant un peu (beaucoup) désordonné et sans base d'écriture scientifique. Merci de m'avoir transmis ta rigueur scientifique, que ce soit pour planifier et organiser un emploi du temps pendant les manips, préparer un protocole expérimental robuste, ou encore lors de l'écriture d'articles. Sans cette organisation, je n'en serais sûrement pas là aujourd'hui.

Merci à Frédéric Jean (directeur de l'IUEM) et Luis Tito de Morais (directeur du LEMAR) pour leur accueil dans le laboratoire. Un merci tout particulier pour votre soutien lors de la période un peu plus difficile durant la deuxième année de thèse, mais aussi lors de mon rapatriement « en urgence » des US fin mars 2020 au début de la crise Covid. Merci également aux responsables de l'équipe Discovery : François Le Loc'h et Olivier Gauthier.

Merci aux membres de mon comité de thèse Fred Olivier et Ronan Fablet. Et un (grand) merci aussi à Flore Samaran d'avoir été présente lors du CSI de deuxième année, qui aura été la partie la plus difficile (mentalement) de ces trois ans. Et merci Flore pour les barbeucs qui faisaient rencontrer les membres (bio)acousticien(ne)s de l'ENSTA et de l'IUEM.

J'ai eu la chance pendant ma thèse d'effectuer de nombreuses plongées scientifiques, que ce soit pour attraper des langoustes ou pour faire des manips d'enregistrements sonores. Je souhaite remercier chaleureusement toute l'équipe de plongeurs de l'IUEM : Erwan Amice, Thierry Le Bec et Isabelle Bihannic. Merci pour toute votre aide, vos conseils techniques et astuces lors de la préparation pré – pendant – post plongée. Merci aussi d'avoir accepté tous mes petits « caprices de biologiste », notamment quand je vous ai demandé de m'aider à installer en plongée en Mer d'Iroise une antenne

linéaire de 100 mètres de long avec 10 hydrophones (placés au centimètre prêt !) pour quantifier la propagation des sons des langoustes ☺ Un merci particulier à Erwan pour ta pédagogie, tes conseils et enseignements sur les rudiments de la plongée scientifique, ainsi que pour tes magnifiques photos et vidéos qui ont sublimé mes powerpoints lors des conférences internationales et pour les articles de journaux mais aussi pour ce manuscrit de thèse. Je n'oublie pas de remercier les autres personnes qui sont venues plonger avec moi pour dépanner : Emilie Grosjean, Julien Thébault, Pierre Poitevin et Guillaume Bridier. Une mention spéciale pour Guillaume qui avait accepté de venir plonger pour échantillonner des langoustes en plein mois de Mars au large de la Mer d'Iroise dans une grosse houle de Noroit (pas sûr que ton ventre accepte de nouveau ce supplice). Merci aussi à Jacques Grall et Laurent Chauvaud pour avoir été barquero quand on manquait de main d'œuvre. Merci enfin aux marins de l'Albert lucas pour les sorties en rade.

J'ai passé la première moitié de la thèse à effectuer de nombreuses expérimentations dans la salle photo du Pavillon tropical d'Océanopolis, et qui ont quand même abouti à la publication de trois articles scientifiques (Chapitres 1, 2 et 4 de ce Manuscrit). Un grand merci à toute l'équipe d'Océanopolis : Céline, Anne, Dominique, Jean, Lionnel, Jean marie, Nicolas, Yann, Sébastien... Un merci particulier à Jean Marie pour les (nombreuses) heures passées à discuter de chasse sous marine, ce qui me permettait de m'évader un instant quand je passais la journée enfermée dans la salle de manip. Merci aussi à Lionnel pour tes mues de juvéniles de homards (que je garde précieusement), et pour les moments passés à discuter « homard ». Merci à l'équipe de bricolage pour leur aide technique et le matériel prêté. Merci enfin aux gardiens qui me permettaient de rentrer et sortir d'Océano à toute heure, même le Dimanche à 23h00. J'espère avoir l'occasion de vous présenter nos travaux lors d'une conférence à Océanopolis une fois que cette pandémie sera derrière nous.

Si il y a bien une personne extérieure au projet sans qui cette thèse n'aurait pas pu se dérouler, c'est Pierre Poitevin (alias Poitevek dans le métier). En effet, ce qu'on oublie souvent quand on lit un article scientifique, c'est que la première étape (qui est donc cruciale pour la suite de l'étude) consiste à prélever ses modèles biologiques (et c'est souvent la partie la plus délicate, même si elle ne tient qu'en quelques lignes dans un article). Merci pour tout Pierre. Tu ne rechignais jamais à venir m'aider à prélever des homards et langoustes, notamment pendant les tempêtes en plein hiver (et que je n'avais qu'un paire de chaussette en guise de chaussons). On a également fait moult sessions (impossible de les compter...) de chasse sous marine, de pêche à pieds et de pêche à la ligne dans nos « secret spots », de jour comme de nuit, en été comme en hiver. Ces sorties me rendent nostalgiques tant elles sont remplies d'anecdotes (il faudrait un livre entier pour les retranscrire). D'ailleurs, j'en profite au passage pour remercier la SNSM du Conquet pour leur rapidité d'action et leur gentillesse (et encore désolé pour la frayeur et le dérangement occasionnés). Merci aussi à Marion pour nous avoir laissé sortir aussi souvent, et encore désolé pour les (nombreuses) fois où on rentrait en retard. J'ai hate de voir Malo reprendre le flambeau.

Merci à toute l'équipe administrative de l'IUEM et du LEMAR : Anne-So, Gene, Yves, Céline, Lionel. Merci également à l'administration de l'EDSML pour leur grande pédagogie face à des administratophobes comme moi : Elisabeth et Elodie.

Je souhaite aussi remercier les enseignants-chercheurs et professeurs de l'UBO et IUEM qui ont su transmettre leur passion pendant les cours de biologie marine de licence et de master : Julien Thébault, Frédéric Jean, Jean laroche, Jacques Clavier...

Merci à Eric Dabas pour ton aide technique lors du stockage et de l'acclimatation de toutes mes bestioles (homards et langoustes, entre autre...) dans les salles climatiques de l'IUEM.

Merci à la DDTM pour leur confiance accordée dans les autorisations de prélèvements de langoustes en plongée en 2018 et 2019.

Merci à Philippe Elies, de la plateforme de microscopie de l'UBO, pour nos dizaines d'heures passées à photographier au MEB les pièces de production sonore des langoustes. Mes yeux s'en rappellent encore ! ☺

Merci à Isblue (ancien Labex mer) et à l'UBO pour les différentes bourses qui m'ont été accordée pour pouvoir partir en conférences (Montréal, Victoria, San Diego) et en séjours à l'international (Gallway et Woods Hole).

Merci à Katel (pnbi) pour ton aide logistique, et pour nous avoir prévenu à chaque fois qu'un pot se terminait au rez de chaussé pour pouvoir récupérer les restes.

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Merci aux thésard(e)s et stagiaires pour les bons moments passés aux pauses café et pendant les vendredi midi « spécial barbecue » : Pierre, Guillaume, Thomas, Fanny, Matthieu, Anatole, Pauline (un jour je battrais ton record de lotte), Valentin (désolé de t'avoir mis des taules au badminton pendant toutes ces années), Arthur, Ho (mais c'était pas sympa de nous avoir balancé pour les pauses cafés, je n'en dirai pas plus), Rémi, Gaetan, le chef Johann (merci pour tes gateaux basques)... Merci aussi à Fred, Jean Alix et Jean-Marc pour nos discussions.

Un merci particulier à Guillaume, collègue de bureau et « pote de galère » durant ces trois ans. Merci pour toutes les soirées passées entre le Mc Guigan's et le Tara Inn (dont nous étions les principaux actionnaires à l'époque), et pour les karaokés sur grand écran. Merci aussi de m'avoir hébergé lorsque ma kangoo pêche était en panne, et de m'avoir sauvé la mise quand j'étais embourbé vers Plouzané à quelques minutes d'une réunion importante...

Je souhaite aussi remercier les (gros) homards qui m'ont tenu compagnie pendant une bonne partie de la thèse, que ce soit dans mes bacs à Océanopolis ou à l'IUEM : Sartorius (le seul et unique),

Spartacus, Kerhornus, Feunteuneus, Kermorvus 1^{er} du nom et Kermorvus 2^{ème} du nom, Clavierus, Moltus, Tréganus.

Merci aux thésard(e)s qui ont grandement contribué à la bonne ambiance lors de la semaine de conférence pour la biodiversité marine à Montréal en 2018 : Guillaume, Pierre, Pauline, et merci aussi à Olivier Gauthier pour cette tournée des (meilleurs) bars de la ville. Merci à Xavier Mouy pour m'avoir proposé de venir à la conférence de l'ASA à Victoria suite à nos discussions à Montréal (et merci de m'avoir permis de remporter un prix à la conférence de l'ASA à Victoria ensuite ☺).

Merci à Rémi et aux collègues de la DGA pour les moments passés à la conférence de l'ASA de Victoria en 2018 (enfin surtout dans les bars). Un énorme merci également au réceptionniste d'un des grands hotels de Victoria pour m'avoir chaleureusement accueilli et offert un café au coin du feu à 3h du matin lors de mon arrivée en ville en pleine tempête (et après 22h sans dormir) et que je perdais espoir à arpenter les rues à la recherche de mon RBB sans portable (et dont je ne me souvenais plus de l'adresse).

Merci à Gaetan et Maelle pour les super moments passés à la conférence de l'ASA à San Diego en décembre 2019, notamment à siroter des margaritas devant les plages de sable blanc au soleil... Dommage que nous n'ayons pas pu surfer à cause du temps à la fin de la conférence, on se rattrapera une prochaine fois !

Merci à RTSYS pour leur soutien technique de dernière minute quand les enregistreurs ne marchaient pas en pleine manip. Un merci particulier à Didier Clec'h pour les discussions « langoustes », et pour m'avoir mis en relation avec le club de Plongée d'Audierne.

Merci à Nicolas Gabaron du club de Plongée d'Audierne pour nous avoir emmené plonger dans vos hot spots à langoustes dans la Baie, et pour nous avoir permis d'enregistrer leurs sons. C'étaient deux super plongées, même si je ne me souviens plus trop de la première (vive la narcose...), heureusement qu'Erwan et Thierry étaient là pour assurer l'installation du matos.

Merci à Emma et aux musiciens de la Carenne qui ont contribué aux échanges art et science : Maxime, François et Vincent. Cela m'a permis de développer ce côté vulgarisation scientifique qui me manquait. Merci aussi à Liz Hascoët pour tes (magnifiques) dessins de homards (dont un qui illustre le début de ce manuscrit).

Merci à Martial Laurans pour nos discussions « langoustes », j'espère avoir l'occasion de plonger avec toi pour aller les voir en Mer d'Iroise (je suis chaud pour l'Ar Men).

Les buzz des homards ont (contre toute attente) attiré l'attention de nombreux(ses) journalistes, qui ont pu retranscrire les résultats de nos recherches sous diverses formes, que ce soient des articles de journaux ou même des émissions vidéos et radios. Ces échanges ont permis de grandement améliorer

mes capacités de vulgarisation scientifique (qui étaient proches de zéro en début de thèse). Merci à Catherine Le Guen (Télégramme), Damien Le Délézir et Gaël Hautemulle (Ouest France), Nathaniel Herzberg (Le Monde), Sciences Ouest, Tanguy monat et Simon Cohen (Bretagne Magasine), France 3 Littoral, Gael Gueguen et son équipe (radio France Bleu Breizh Izel ; maintenant je sais que le homard se « Cousteau »)...

I am now going to thank (in english of course) all the wonderful people I've meet during my trips in Galway and Woods Hole.

My first Ph. D. trip was in Ireland (september 2018), which is similar to Brittany but with wilder nature, as well as with more lobsters (I checked myself). Many thanks to Martin Gammel and Ian O'Connor for hosting me during one week in their amazing marine biological laboratory in GMT (Gallway). Thank you Martin for letting me participate to your classes on behavioural analysis. Also thank you for letting me give a seminar of 20 mins of our lobster researches in front of GMT students and researchers, which was a first challenge for me, and many thanks to Ian for rewarding me with wonderful Irish beers ☺. (Un)fortunately, a big storm prevented me to come back in France at the end of the week, and I had to stay four more days where I visited South Ireland. Thanks to the amazing family who hosted me in their bed and breakfast's house during this time.

I have had the great opportunity to spend two trips (thanks to Julien) as a guest student at the Woods Hole Oceanographic Institution (Woods Hole, US) in 2019 (January-February) and 2020 (January-March), and I want to thank all the great people I have had the chance to meet.

First, I thank all the administration staff for their great help, notably Amanda and Gretchen for their kindness. Thanks to Dennis McGillicuddy for welcoming me at the Applied Ocean Physics and Engineering Department lab in 2019, and thanks for letting me present our lobster bioacoustic work in front of the acoustic gods, it was a great moment! And thanks to Tim and Dan for our discussions on particle motion. Also thanks to Annamaria for the NOAA-MBL seminar in MRF.

Thanks to Jean and Megan for hosting me in their big and beautiful house in Fern Lane during these two years.

A giant thank to all my roomates for your kindness and all the parties we did together in the house and elsewhere. I was very lucky to have you there in 2019 and 2020: Emmanuel, Marissa, Alexander, Suzy, Rajesh, Zaho, Jasmin and Luis. I do not know how to thank you Emmanuel for everything you did (you are the best cook ever), but I do know one thing: I cannot wait to come back to finish all the things we planned to do (Martha's Vineyard, ski, New York...). Alexander, I would love to visit your office in Harvard next time. Marissa, I hope we'll play more games together (I promise I won't be cheating anymore...) and parties, as well as snorkeling to catch lobsters next time. I was so sad I had to leave in a hurry due to the Covid crisis in 2020, but we will catch up ☺

Thanks to all the students for the amazing parties and dinner nights during the (long) winter nights in WHOI: Seth, kayla, Samantha, Sarah, Manyu, Emily, Christiana, Ian, Hansen, Gaetan, Laura... I hope to see you again ☺ Thanks to Seth for driving us everywhere, to play football and for shopping in Falmouth, for climbing or going to the bars!

Thanks to Coffee O and the MBL cafeteria for providing me with enough caffeine and food to stay alive during these two hard work periods ☺

I had the privilege to come back in WHOI in 2020 to perform bioacoustic experiments with American lobsters in Aran Mooney's lab at the Marine Biological Lab. Thank you very much Aran for hosting me and letting me use your experimental equipment. I really loved working in your lab. I still feel sad I had to leave in a hurry late March 2020, and we did not have the opportunity to make the big lobster dinner party we planned to do. I really hope I will come back as a post doc and continue to work with you (either on squids or lobsters).

Thanks to Aran Mooney's lab people for your help and kindness (I know how hard it is to talk daily to a french guy with a poor english accent): Ian, Seth, Frants and Andria. A huge thank to Ian for his (amazing) help during lobster experiments, even if you also had to work on your Ph. D. project at the same time. Thanks to Frants for all our discussions on bioacoustics, and for the experiments we planned to do with lobsters, I hope we'll be able to perform them when I'll come back ☺

Thanks to all the shuttle bus drivers and WHOI guardians for their kindness and for all the rides between the Village and Quisset Campus late at night.

Thanks to Arthur Popper who introduced me to Jelle Atema after the ASA meeting in Victoria (2018) where I presented our work on lobster acoustic behaviour.

When I came to Woods Hole, I would have never expected to meet and talk to the American lobster god: Jelle Atema. In fact, all the scientific litterature based on lobster behaviour and chemical communication since 50 years has been written by this researcher, and they were the basis of our work on European acoustic behaviour in Brittany. Thank you Jelle for our wonderful discussions about lobsters, and for providing us all your scientific knowledge during neurophysiological experiments in 2020. It is a great honour to have you as co-author in our scientific paper that came out from these experiments. I hope I can come back so I can taste again you wonderful home-made lobster pasta!

I also had the chance to talk to the American lobster goddess: Diane Cowan. Thanks Diane for our discussions on lobster behaviour. I think I've meet someone who is even more passionnate about these creatures than I am (which I did not think it was even possible). Thanks for you interests and participation to the NOAH lobster proposal we wrote together in WHOI with Julien, Aran and Alex. I hope we will have another opportunity to resubmit it, and that we could finaly meet and make some experiments on your wonderful island.

Revenons maintenant à Brest et ses alentours.

Merci à tous les collègues du badminton club des Manchots de la Rade pour ces 8 années (déjà...) à passer la serpillère à Kerabram. Merci à Gomez pour les séances interminables de simple, et à Mathieu Le Coguic pour tous les matchs, entraînements et tournois de double qu'on a pu faire ensemble. Se défouler sur des volants après une grosse journée de boulot, ça fait toujours du bien ☺

Merci à Gildas et Christophe pour les sorties chasse sous marine en dérive à Ouessant, qui changeaient des sorties du bord. Descendre au pied du phare de la Jument ou dans la Chaussée de Keller ont été des moments magiques tellement ces lieux sont remplis d'histoires (même si c'est pas évident de descendre sur des têtes de roche dans un courant de dingue en sachant que le fond autour avoisine les 60 m).

Merci à Maxence Gémin, mon merveilleux binôme de chasse sous marine en dérive dans la baie de Perros Guirec (mon jardin). On en a passé des week ends à traquer le lieu et le bar dans nos spots... Il ne manque que le spot à grosses langoustes (que je garde encore secret) et tu connaîtras tout mon jardin par coeur ☺ Et merci aussi à Florian pour les sorties chasse sous marine, et pour les cueillettes de champignons.

Merci aux copains et copines du Master pour les soirées et moments passés ensemble (en présentiel ou en skype, crise covid oblige): Marion, Morgan, Maxence, Quentin, Raf...

Merci au chef Gueno, Orlanouille et Louis pour tous les bons moments passés ensemble depuis nos débuts à la fac, même si on s'est un peu perdu de vue sur la fin de la thèse (la pandémie n'aidant pas vraiment...).

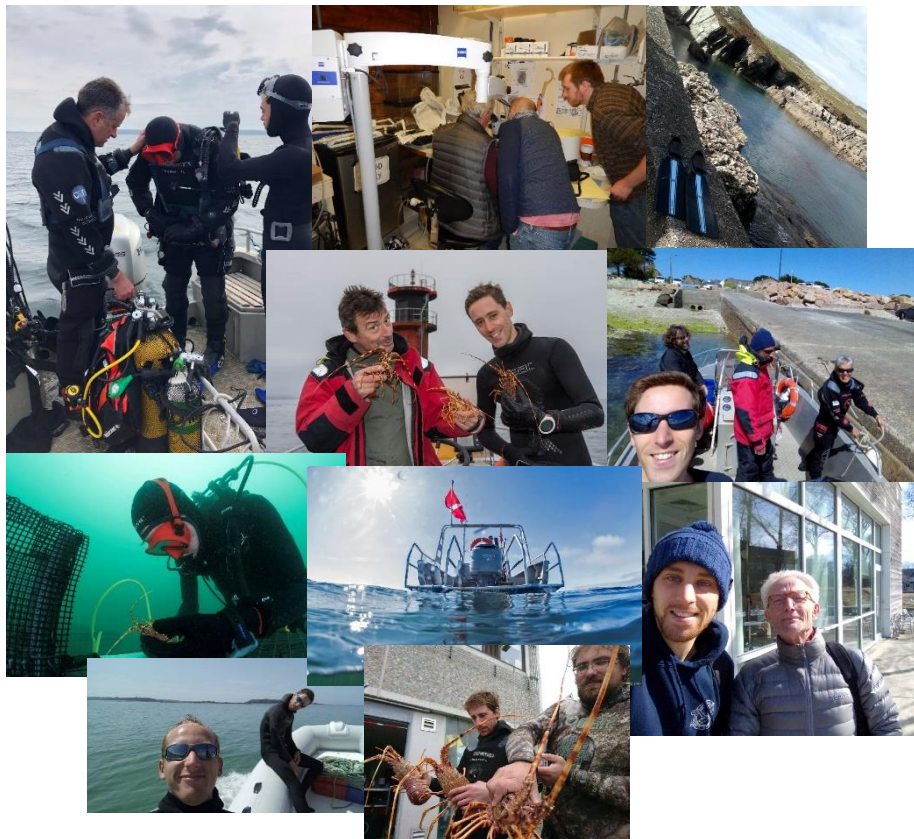
Merci aux copains du lycée pour les week ends endiablés à jouer aux jeux de sociétés (Donjons et Dragons, entre autre...): Rob, Clément, Rémi, Mymy...

Les connaissances empiriques sur le milieu marin, notamment sur le comportement des espèces, se font principalement via des observations directes par le biais de la pêche. Comme disait Jacques-Yves Cousteau, « le meilleur moyen d'observer un poisson est de devenir un poisson ». J'ai la chance d'être entouré depuis tout petit par des dieux de la pêche, qui m'ont tant appris sur le comportement des crustacés et des poissons. Merci à Anthony (le seigneur des labrax) pour les sorties chasse aux bars en fin de journée. Merci à Eric pour toutes les parties de pêche, je pense par exemple aux sorties nocturnes « spécial confinement »... Merci également à Thomas pour m'avoir fait découvrir les montagnes Ariégeoises lors de parties de pêche à la truite dans des lacs en haute altitude, ainsi que pour les sorties pêche au carnassier (mon record de sandre est toujours d'actualité). Ca m'a fait du bien l'air de la montagne pour me vider la tête au milieu de la thèse ☺ Merci aussi à Rico et Monique pour les nombreux moments passés sur l'eau et lors des repas à déguster notre godaille.

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Introduction générale

L'importance du son chez les animaux marins

L'océan est bien loin du Monde du Silence présenté par Jacques-Yves Cousteau en 1956. Cet environnement est non seulement riche en signatures sonores, mais il est aussi établi que le son est un signal essentiel et largement utilisé par les organismes marins pour transmettre et recevoir des informations (Tyack 1998). Ces animaux tirent profit des propriétés physiques du son dans l'océan. Par exemple, les sons se propagent environ 5 fois plus vite dans l'eau que dans l'air (Urick 1983). Les écologistes voient donc dans ces signaux acoustiques le moyen pour certaines espèces de compenser les contraintes environnementales propres à l'océan. Par opposition aux signaux chimiques et visuels, les signaux acoustiques sont détectables sur de longues distances lorsque la visibilité est nulle, la nuit, ou réduite du fait de la turbidité en zones oligophotiques et aphotiques. Ces signaux se propagent indépendamment des courants et contiennent des informations directionnelles fiables. Ainsi, les animaux marins ont la possibilité d'utiliser ces propriétés sonores, et les sons doivent être vus comme faisant partie du panel d'informations environnementales qui leur est disponible pour diverses raisons écologiques. Il n'est pas absurde d'imaginer que l'évolution a permis des relations écologiques centrées sur ces sons, entre les organismes eux-mêmes (vertébrés et invertébrés), et entre les organismes et leur environnement. Ainsi, la bioacoustique, qui s'intéresse aux sons produits par les organismes, trouve d'abord sa motivation dans ces études autoécologiques.

De façon évidente, les sons émis par les mammifères marins sont nettement les plus décrits dans la littérature bioacoustique (Richardson et al. 1995, Tyack and Clark 2000). Toutes les espèces de mammifères marins (pinnipèdes, odontocètes, mysticètes) sont connues pour émettre des sons afin de communiquer, s'alimenter ou encore se déplacer et s'orienter. La plupart de ces sons est générée via des mouvements d'air à travers différents tissus (Frankel 2009). Chaque espèce possède son propre répertoire vocal dont la fréquence, la complexité et la variabilité dépendent du contexte social (Au and Hastings 2008). Par exemple, les baleines

bleues émettent des chants basses fréquences (< 100 Hz) et très intenses pour communiquer entre individus d'un océan à l'autre (Sirovic et al. 2007, Samaran et al. 2010, Leroy et al. 2018). Les baleines franches produisent de longues chansons pendant la période de reproduction entre mâles et femelles pour se trouver et se choisir comme partenaires (Clark 1990, Edds-Walton 1997). Les dauphins et baleines à dents utilisent également l'écholocation en émettant des cliquetis hautes fréquences (jusqu'à plusieurs centaines de kilohertz) pour s'orienter dans les fonds marins et pour détecter leurs proies à la manière d'un sonar actif (Au 2012, Miller et al. 2004, Madsen et al. 2005a).

Les connaissances acoustiques sur les poissons sont moins complètes et plus récentes que celles accumulées pour le groupe des mammifères marins, mais tendent à augmenter largement dans la littérature bioacoustique depuis le début des années 2000 (Ladich 2015). En effet, la phonotèque ichthyologique répertorie désormais les sons émis chez les poissons pour près de 1000 espèces sonifères (Luczkovich et al. 2008a), et on estime à dix fois plus leur nombre réel (i.e. soit un tiers du nombre d'espèces total de poissons ; Ladich and Bass 2011). Ces sons sont produits par deux principales catégories de mécanismes : la vibration musculaire de leur vessie natatoire, et la stridulation via le frottement de pièces osseuses entre elles (Ladich 2014, Parmentier and Fine 2016). La plupart de ces sons se situe dans les basses fréquences (inférieures à 1 kHz), que les poissons peuvent détecter grâce à leur oreille interne ou leurs lignes latérales (Popper and Fay 2011). Ces sons sont utiles pour de nombreuses fonctions écologiques, que ce soit lors de communications intra ou inter-spécifiques. Par exemple, le poisson clown claque des dents pour éloigner des intrus de son territoire (Parmentier et al. 2007), le grondin gronde à l'approche d'un prédateur (Connaughton 2004), le mérrou grogne pendant la parade nuptiale (Nelson et al. 2011), et le mâle poisson crapaud siffle pour attirer une femelle dans son terrier lors de la période de reproduction (Gray and Winn 1961).

En comparaison, le rôle écologique des sons chez les invertébrés benthiques, en particulier les crustacés, reste à décrire précisément (De Soto 2016, Edmonds et al. 2016). Cette lacune dans la communauté des écologistes marins est sans nul doute liée aux carences dans l'état général de nos connaissances sur leur écologie.

Quel est le rôle écologique des sons chez les crustacés marins ?

Toutefois, on sait depuis plusieurs décennies que certaines espèces de crustacés émettent des sons à l'aide d'une grande diversité de mécanismes (Schmitz 2002). Par exemple, les rasps* d'antennes produits par les langoustes (Palinuridae) sont documentés depuis plus de 1500 ans (Athenaeus 300). Ils sont générés par un mécanisme de stridulation similaire à ceux des arthropodes terrestres (e.g. criquets), et seraient produits dans une action de défense, principalement dans l'intention de faire fuir des prédateurs (Moulton 1957, Patek 2001, Bouwma and Herrnkind 2009). A l'inverse, la crevette-pistolet (Synalpheidae, Alpheidae), crustacé dont les sons sont largement décrits, produit une impulsion provoquée par l'implosion violente d'une bulle de cavitation en claquant sa pince proéminente (Versluis et al. 2000). Le son émis est si intense qu'il paralyse les proies et prédateurs à proximité (Au and Banks 1998). Curieusement, ce son apparaît dans toutes les publications décrivant des paysages acoustiques benthiques alors que le groupe n'est pas ubiquiste. Enfin, le homard Américain et la crevette mante tropicale vibrent leur carapace pour produire des sons basses fréquences appelés buzz* ou rumbles* dont le but serait de faire fuir les prédateurs (Patek and Caldwell 2006, Ward et al. 2011). Les études portant sur la bioacoustique des crustacés le long de la façade Atlantique restent rares, bien que de récents travaux aient démontrés, de façon inattendue, qu'un grand nombre d'espèces de crustacés en Bretagne (e.g. araignée) sont capables de produire des sons (Coquereau et al. 2016a, 2016b). Les fonctions écologiques de ces émissions sonores, si elles existent, restent à définir.

*Nous avons choisi d'utiliser les anglicismes de ces différents sons dans le Manuscrit.

Les quelques exemples présentés ci-dessus montrent que les crustacés, s'ils sont capables d'émettre des sons, semblent limiter leur utilisation aux relations proie-prédateur. Le rôle de ces sons pour la communication intra-spécifique n'est pas encore décrit. De plus, nous ne disposons pas d'informations sur les comportements acoustiques (i.e. émission d'un son pendant un comportement particulier et/ou réponse comportementale à un son) de ces animaux. Il convient toutefois de noter que quelques études démontrent la production de sons entre individus de la même espèce, comme les bernards l'hermite et les langoustes tropicales durant des rencontres agonistiques entre mâles (Mulligan and Fischer 1977, Briffa et al. 2003). Cependant, la communication intra-spécifique, si elle est envisageable, est toujours en questionnement car on ne connaît pas les capacités sensorielles de ces espèces vis-à-vis de ces sons (Popper et al. 2001). Autrement dit, nous ne connaissons pas les capacités auditives des crustacés marins. En contraste marqué, les crabes semi-terrestres (Ocypodidae) sont non seulement capables de produire des sons, mais ils peuvent aussi répondre à ces sons durant des interactions intra-spécifiques, comme lors de la période de reproduction (Crane 1966, Horch and Salmon 1972, Horch 1975).

C'est dans ce contexte que se pose la question des récepteurs sensoriels chez les crustacés marins susceptibles d'utiliser les sons. Aujourd'hui, trois différents types de récepteurs sensoriels, externes et internes, ont été répertoriés dans la littérature bioacoustique. Tous les trois pourraient être sensibles aux sons basses fréquences chez les crustacés (< 1 kHz ; Cohen and Dijkgraaf 1961, Bush and Laverack 1982, Budelmann 1992). La première catégorie de récepteur inclut les systèmes de récepteurs superficiels englobant les poils sensoriels qui couvrent leur cuticule externe. Ces poils auraient la même fonction que la ligne latérale des poissons et permettraient donc de détecter les mouvements d'eau induits par des sons (Laverack 1962, Popper et al. 2001). Le second type de récepteur correspond aux organes chordotonaux qui sont des cellules sensorielles associées aux parties flexibles des appendices et pourraient

détecter des vibrations (Budelmann 1992). Enfin, l'organe le plus étudié est le récepteur sensoriel interne appelé statocyste, situé dans le segment basal des antennules (Sekiguchi and Terazawa 1997). Il s'agit d'une chambre remplie de fluide contenant des grains de sable, le statolithe, qui est en contact avec des poils sensoriels (Popper et al. 2001). Ce récepteur agit comme un accéléromètre et son rôle est principalement attribué à l'équilibre, mais il pourrait également être sensible aux sons basses fréquences (Lovell et al. 2005, Radford et al. 2016).

Ainsi, à quelques rares exceptions près, nous disposons de peu d'informations sur la bioacoustique des crustacés marins en général, que ce soit en terme de comportement acoustique ou de biologie de l'audition. Ces informations écologiques sont néanmoins cruciales à déterminer dans le contexte actuel où les bruits d'origine humaine, dont l'intensité est croissante dans les océans, ont le potentiel d'impacter toute la faune marine.

Le bruit d'origine humaine, une nouvelle source de pollution dans les océans

Si le son est bien considéré comme une variable essentielle dans l'océan, comprendre son rôle écologique chez une espèce est nécessaire pour appréhender les potentiels impacts des bruits liés aux activités humaines, englobés sous le terme d'anthropophonie (Popper and Hawkins 2014). Parmi les différentes sources sonores, on liste entre autre la navigation des bateaux (de plaisance et de commerce mais aussi la pêche), la prospection sismique (via des canons à air) pour la recherche de pétrole dans le plancher océanique, ou encore le battage de pieux et le forage lors des constructions. Ces différentes sources sonores dominent particulièrement les basses fréquences (< 1 kHz ; Hildebrand 2009), ce qui correspond à la bande de fréquence utilisée par la plupart des organismes marins pour communiquer (Slabbekoorn et al. 2010).

Jusqu'à ce jour, la majorité de la littérature bioacoustique étudiant les impacts des bruits anthropiques sur la faune marine s'est majoritairement focalisée sur les mammifères marins et les poissons (Madsen et al. 2006, Hawkins et al. 2015). Chez ces deux groupes d'animaux, les

impacts identifiés vont du masquage acoustique des communications (Clark et al. 2009, Radford et al. 2014, Neenan et al. 2016) à la mort des individus (Wright et al. 2007, Kunc et al. 2016), en passant par des modifications comportementales ou physiologiques (Nowacek et al. 2007, Popper et al. 2003, Mills et al. 2020). Parmi les invertébrés marins, les céphalopodes ont reçu le plus d'attention vis-à-vis de l'impact des bruits anthropiques. Diverses études ont montré le développement de comportements d'alarme chez les calamars et seiches, ce qui affecte leur communication (Samson et al. 2014, Jones et al. 2020). De plus, des bruits anthropiques intenses induisent des traumatismes acoustiques avec des conséquences irréversibles sur les structures responsables de leur sens d'équilibre et de position (André et al. 2011, Solé et al. 2017).

Dans le cadre de ce travail doctoral, il convient de noter que les études portant sur l'impact de l'anthropophonie sur les crustacés marins sont peu nombreuses principalement du fait du manque de compréhension du rôle écologique de leurs sons (De Soto 2016, Edmonds et al. 2016). Toutefois, les efforts de recherche sur ce champ disciplinaire tendent à augmenter depuis une décennie (Williams et al. 2015). Par exemple, nous savons que les bruits de bateau perturbent le comportement alimentaire chez le crabe vert et ralentissent sa réponse face à des prédateurs (Wale et al. 2013). Ces mêmes sons anthropiques altèrent également les comportements sociaux de groupe chez le bernard l'hermite (Tidau and Briffa 2019). Ils affectent aussi la physiologie des crustacés en modifiant leurs paramètres hématologiques (Filliciotto et al. 2014, 2016). Le bruit de battage de pieu provoque également des comportements de fuite chez le bernard l'hermite (Roberts et al. 2016, Roberts and Laidre 2019). A contrario, aucun impact comportemental et physiologique des bruits de canons à air (classés parmi les plus intenses dans les océans) n'a été reporté sur le crabe des neiges, le homard américain et la langouste australienne (Christian et al. 2003, Payne et al. 2007, Day et al. 2016). Il est important de noter que la plupart de ces études ont été effectuées en cuve où la

forme et la propagation des sons sont fortement affectées (nous reviendrons sur ce point dans les chapitres 1, 2 et 4), et l'extrapolation au milieu marin est rendue particulièrement délicate (Carroll et al. 2017). Ainsi, ces études d'impact restent à définir, notamment dans un contexte national actuel où les projets de construction de champs éoliens en mer (qui génèrent des sons intenses dans le milieu marin) rendent cette information vitale pour la conservation des populations de crustacés.

L'anthropophonie est désormais reconnue comme une source de pollution majeure dans l'océan (National Research Council 2003). Les réglementations nationales et internationales sont en cours d'élaboration, et varient considérablement d'un pays à l'autre (Erbe 2013). L'avancée la plus significative est probablement la directive-cadre sur la stratégie pour le milieu marin menée par l'Europe. Les états membres, dont la France, doivent atteindre et maintenir un bon statut environnemental, comme mesuré par 11 descripteurs, dont un se focalisant sur les bruits anthropiques. Le terme pollution sonore y est défini comme « une introduction directe ou indirecte dans le milieu marin, par suite de l'activité humaine, (...) de sources sonores sous-marines d'origine anthropique, qui entraîne ou est susceptible d'entraîner des effets nuisibles pour les ressources vivantes (...) ». C'est dans cette situation réglementaire que nous pouvons placer la présente étude, car il est possible d'utiliser les crustacés en position centrale lors des études d'impact de la pollution sonore sur la faune benthique. En effet, ces derniers représentent des maillons essentiels au fonctionnement des écosystèmes (Boudreau and Worm 2012), et des pêcheries importantes pour l'homme (Phillips 2008).

En amont des études d'impact et de la gestion des écosystèmes côtiers, l'étude des sons par acoustique passive peut être envisagée comme une voie de recherche pour mettre au point de nouveaux descripteurs de l'état de santé de l'environnement. Le suivi des crustacés, basé sur l'écoute de leurs sons dans le milieu marin, constitue ainsi une autre piste de travail.

L'acoustique passive pour suivre les crustacés dans leur environnement

En effet, en plus d'étudier le rôle écologique des sons produits par une espèce, ceux-ci pourraient également nous fournir un outil pour l'étudier directement dans son milieu, ce qui représente un défi classique en écologie marine. C'est le cas pour les crustacés, dont les outils traditionnels pour estimer leur abondance, leur diversité ou encore leurs mouvements buttent sur de nombreux défis techniques et éthiques. La plongée est souvent utilisée comme moyen d'observation discret pour estimer l'abondance d'individus, mais est limitée par la faible visibilité et le court temps passé sous l'eau (Karnofsky et al. 1989, Breen and Booth 1989). De plus, le caractère nocturne ainsi que la petite taille de la plupart des crustacés les rendent difficiles à observer en journée, et leur diversité et abondance tendent à être systématiquement sous évaluées (Brock 1982). D'autres techniques utilisent le capture-marquage-recapture et des marques télémétriques pour suivre leurs mouvements (Bernardez et al. 2005, Giacalone et al. 2006, Hunter et al. 2013). Cependant, ces deux méthodes nécessitent de prélever les individus d'étude pour les marquer, ce qui génère un stress post marquage qui est rarement évalué mais doit cependant être pris en compte dans l'analyse des données. L'effort d'échantillonnage est également important pour prélever, marquer et recapturer les individus, ce qui tend à focaliser ces observations sur de petites surfaces d'étude. De plus, les crustacés grandissent par mues successives et peuvent évidemment perdre les marques durant leur croissance (Gonzalez-Vicente et al. 2012). Enfin, des estimations de densité de population sont aussi effectuées en utilisant des filets droits ou chaluts de fond (Tuck et al. 1997, Goni et al. 2003, Chen et al. 2005). Ces méthodes de capture, qui utilisent des engins fortement destructeurs de leur habitat, sont pour le moins invasifs pour l'espèce étudiée. Citons ici les travaux de Catanese et al. (2018) qui démontrent que seulement 64% des juvéniles de langoustes capturés au filet et relâchés survivent. Il est donc essentiel de développer de nouveaux outils d'étude non invasifs et non destructeurs pour suivre les populations de crustacés dans leur environnement.

Le suivi par acoustique passive se base sur l'écoute des sons produits par les espèces dites sonifères (Rountree et al. 2006). Elle s'applique donc aux crustacés. Cette méthode de suivi discrète et non intrusive n'a pas d'impact sur la faune marine et permet d'enregistrer en continu (de quelques heures à plusieurs années) et à bas coût les sons, indépendamment des conditions météorologiques et des différences de luminosité jour-nuit (Sousa-Lima et al. 2013). Ainsi, cet outil est utilisé depuis de nombreuses décennies dans le cas de suivis des mammifères marins et plus récemment chez les poissons, qui émettent des sons détectables dans le milieu marin sur plusieurs dizaines (voir centaines) de kilomètres à quelques mètres, respectivement (Bonnel et al. 2014 ; Locascio et al. 2011). L'acoustique passive présente désormais un large panel d'applications, passant par la détection et l'identification d'espèces cryptiques ou en danger d'extinction (Rayment et al. 2011, Samaran et al. 2013, Picciulin et al. 2019), l'estimation de densité de populations (McDonald et al. 1999, Rowell et al. 2017, Jacobson et al. 2017), la localisation d'individus (Freitag and Tyack 1993, Tiemann et al. 2006, Putland et al. 2018), le suivi des rythmes d'activités (Dede et al. 2014, Wang et al. 2015, Rice et al. 2017) et de la période de reproduction (Luczkovich et al. 2008b, Jublier et al. 2020, Caiger et al. 2020). En plus d'être non-invasif vis-à-vis de l'espèce étudiée et non-destructeur de l'environnement, cet outil offre une description environnementale à large échelle (les sons se propagent sur des kilomètres sous l'eau) et sur de longues périodes (enregistrements en continu sur plusieurs mois).

La littérature bioacoustique montre qu'il y a aujourd'hui très peu d'études utilisant l'acoustique passive pour suivre les crustacés *in situ*. Les seules études disponibles sont faites sur la crevette-pistolet des genres Synalpheidae et Alpheidae, dont les sons impulsifs dominent certains écosystèmes marins tropicaux. En effet, des études ont montré la relation entre le nombre d'impulsions de crevettes-pistolets avec les cycles célestes, saisonniers et diurnes (Bohnenstiehl et al. 2016, Lillis and Mooney 2016, 2018), et l'abondance d'individus

entre des habitats sains et dégradés (Butler et al. 2017), ou encore avec des indices de pollution de l'eau de mer comme la diminution en oxygène (Watanabe et al. 2002). Ainsi, la crevette-pistolet peut être décrite comme sentinelle de l'environnement, sa production sonore pouvant renseigner sur l'état de santé des écosystèmes. En considérant le nombre d'espèces sonifères répertoriées chez les crustacés (voir section 2 ci-dessus), l'acoustique passive semble être un outil très prometteur pour les suivre *in situ*. Il apparaît désormais nécessaire de répertorier les espèces de crustacés à fort potentiel acoustique et d'estimer leurs distances de détection, afin de développer de nouveaux outils d'évaluation de santé des écosystèmes marins.

Objectifs de la thèse et plan du manuscrit

Dans ce contexte général, ce travail de thèse s'est intéressé à deux espèces de crustacés présentes sur les côtes Européennes : le homard Européen *Homarus gammarus* (Linnaeus 1758), et la langouste rouge *Palinurus elephas* (Fabricius 1787).

Objectifs

Ce travail de thèse vise à améliorer nos connaissances sur le rôle écologique des sons chez les crustacés en :

- 1) caractérisant leurs sons en tenant compte des effets physiques liés aux cuves dans lesquelles sont réalisées les expérimentations de laboratoire,
- 2) déterminant leurs comportements acoustiques, et
- 3) appréhendant leurs capacités sensorielles dans la détection de ces sons.

Nous tenterons notamment de répondre aux deux grandes questions suivantes :

- Les sons produits par le homard et la langouste servent-ils de moyen de communication intra-spécifique ?
- Dans quelles mesures les sons qu'ils produisent peuvent être utilisés comme outil de suivi *in situ* par acoustique passive ?

Pour répondre à ces questions, nous avons choisi de suivre, dans un souci de complémentarité, différentes approches en laboratoire (i.e. en aquarium) mais aussi en milieu naturel.

Plan du manuscrit

Ce manuscrit de thèse s'articule autour de deux grandes parties, correspondant chacune à nos deux modèles d'étude. Chaque partie est constituée d'une introduction sur l'utilisation des deux modèles d'études, et de différents chapitres écrits sous la forme d'articles scientifiques visant *in fine* à répondre aux différentes questions posées précédemment. Chaque chapitre comprend également un résumé en français présentant les principaux résultats écrits en anglais dans les différents articles scientifiques. Des transitions entre chaque chapitre, écrites aussi en français, ont été effectuées pour rendre la lecture de ce manuscrit plus fluide. Enfin, le manuscrit se termine par une conclusion générale discutant les principaux résultats, ainsi que des perspectives d'études avec un relief particulier pour l'étude de l'impact des bruits anthropiques sur les crustacés, et de l'utilisation de leurs sons comme suivi par acoustique passive in situ.

Partie 1 : Le homard, un grand bavard ?

Chapitre 1 : Caractérisation sonore du homard Européen en cuve

Le **chapitre 1** vise à caractériser les sons produits par le homard en tenant compte de l'effet physique des cuves sur ces sons. Les résultats de cette étude nous ont servi de référence pour les études suivantes, avec notamment la mise en place d'une méthodologie de caractérisation sonore en cuve selon le type de son produit par les crustacés.

Le chapitre 1 a été publié dans : **Jézéquel Y.**, Bonnel J., Coston-Guarini J., Guarini J.M. and Chauvaud L. (2018). Sound characterization of the European lobster *Homarus gammarus* in tanks. *Aquatic Biology*, 27:13-23. L'article est présenté dans l'Annexe 3.

Chapitre 2 : Comportement acoustique des homards Européens mâles

Le chapitre 1 ayant montré la production de buzz basses fréquences par le homard Européen, et similaire au homard Américain, nous nous intéressons dans le **chapitre 2** au contexte durant lequel ce son est produit. Nous démontrons que ces buzz sont produits par les homards mâles lors de rencontres agonistiques pour établir des statuts de dominance, et soulevons l'hypothèse de leur rôle dans une communication intra-spécifique.

Le chapitre 2 a été publié dans : **Jézéquel Y.**, Coston-Guarini J., Chauvaud L. and Bonnel J. (2020a). Acoustic behaviour of male European lobsters (*Homarus gammarus*) during agonistic encounters. *Journal of Experimental Biology*, 223:jeb211276. L'article est présenté dans l'Annexe 4.

Chapitre 3 : Détection sonore du homard Américain

Le chapitre 2 ne permet pas de valider le rôle des buzz dans la communication intra-spécifique, car on ne connaît pas les capacités sensorielles des homards vis-à-vis des sons. Le **chapitre 3** vise ainsi à développer un audiogramme du homard et déterminer les organes de réception des sons via une approche neurophysiologique. Les résultats démontrent que les homards peuvent détecter leurs propres sons, et renforcent leur rôle dans la communication intra-spécifique. Le chapitre suggère également que l'organe de réception sonore principal serait les poils sensoriels recouvrant leur carapace.

Les travaux du chapitre 3 ont été réalisés durant une visite de 3 mois au Woods Hole Oceanographic Institution (WHOI ; MA, USA), en collaboration avec Aran Mooney. Ce chapitre a fait l'objet d'un article en cours de révision dans *The Journal of Experimental Biology* : **Jézéquel Y.**, Thomas Jones I., Bonnel J., Chauvaud L., Atema J. and Mooney T.A. (202X). Sound detection by the American lobster (*Homarus americanus*).

Partie 2 : La langouste, comme un grillon dans la mer ?

Chapitre 4 : Comparaison de rasps d'antennes émis par la langouste en cuve et *in situ*

Le chapitre 1 a démontré l'effet physique des cuves sur les sons des crustacés, mais la littérature bioacoustique des langoustes n'a caractérisé leurs sons qu'en cuve. Dans le **chapitre 4**, nous avons effectué une comparaison des rasps de langoustes en cuve et *in situ* pour présenter leurs différences sonores. Il en ressort que les sons des langoustes dans le milieu marin sont intenses et avec de l'intensité principalement répartie dans les basses fréquences.

Le **chapitre 4** a été publié dans : **Jézéquel Y.**, Bonnel J., Coston-Guarini J. and Chauvaud L. (2019). Revisiting the bioacoustics of European spiny lobsters *Palinurus elephas*: comparison of antennal rasps in tanks and in situ. *Marine Ecology Progress Series*, 615:143-157. L'article est présenté dans l'Annexe 5.

Chapitre 5 : Caractéristiques sonores de différentes tailles de langoustes dans le milieu marin : niveaux sources, propagation et distances de détection

Le chapitre 4 ayant montré le caractère intense des sons de langoustes, nous avons cherché à estimer leurs distances de détection. Dans le **chapitre 5**, nous avons développé une antenne d'hydrophones de 100 m de long pour quantifier *in situ* les niveaux sources et pertes de transmissions des sons de langoustes de différentes tailles. Ces mesures ont permis d'estimer que les sons des grosses langoustes peuvent être détectés sur plusieurs kilomètres dans le milieu marin, ce qui fait de l'acoustique passive un nouvel outil prometteur pour suivre ces animaux.

Le chapitre 5 a été publié dans : **Jézéquel Y.**, Chauvaud L. and Bonnel J. 2020b. Spiny lobster sounds can be detectable over kilometers underwater. *Scientific Reports*, 10:1-11.

Outre les publications dans des journaux scientifiques précédemment cités, les travaux de thèse ont également été présentés dans des conférences internationales. Les présentations orales des chapitres 2 et 5 ont été récompensées à deux reprises par le prix de la seconde meilleure présentation en 2018 et 2019 (respectivement), décerné par le comité technique bioacoustique de l'Acoustical Society of America.

Partie 1 : Le homard, un grand bavard ?



© Nathalie Bihan (redessiné à partir d'une photo d'Erwan Amice)

Préambule

Le choix du premier modèle d'étude, le homard Européen, a été motivé par diverses raisons. Tout d'abord, cet animal possède une forte valeur patrimoniale et commerciale en Europe, notamment en Bretagne. De plus, nos connaissances sur son écologie et ses comportements restent éparpillées, et aucune étude bioacoustique n'a été rapportée à ce jour sur cette espèce. En contraste marqué, la littérature bioacoustique et comportementale disponible chez le homard Américain, *Homarus americanus*, est beaucoup plus abondante et il nous était possible de nous inspirer de ces travaux.

Production sonore du homard Américain

Dès la moitié du 20^{ème} siècle, Moulton (1957) est le premier auteur à évoquer la possible production sonore par le homard Américain, en décrivant les vibrations de sa carapace lors de la manipulation d'individus. Fish (1966) enregistre une décennie plus tard et pour la première fois avec un hydrophone les sons produits par ses vibrations, qui sont des sinusoides basses fréquences (~ 100 Hz) de plusieurs centaines de millisecondes. Mendelson (1969) découvre ensuite des muscles à contraction très rapide, localisés à l'intérieur de la carapace, qui seraient responsables de la production des sons décrits par Fish (1966). Plus récemment, Henninger and Watson (2005) étudient en détail la production sonore du homard Américain. On sait aujourd'hui qu'à l'intérieur de sa carapace se trouvent deux paires de muscles accrochés antérieurement aux antennes secondaires, et fixés postérieurement à la carapace (Figure 1 A). Lorsque ces muscles se contractent, ils font vibrer la carapace, ce qui génère la production d'un son basse fréquence, appelé buzz, avec les mêmes caractéristiques temporelles et spectrales que les vibrations de la carapace (Figure 1 B). Il convient de noter qu'une seule étude s'est intéressée au rôle écologique de ces buzz. Ces sons serviraient à faire fuir des prédateurs comme des poissons (Ward et al. 2011).

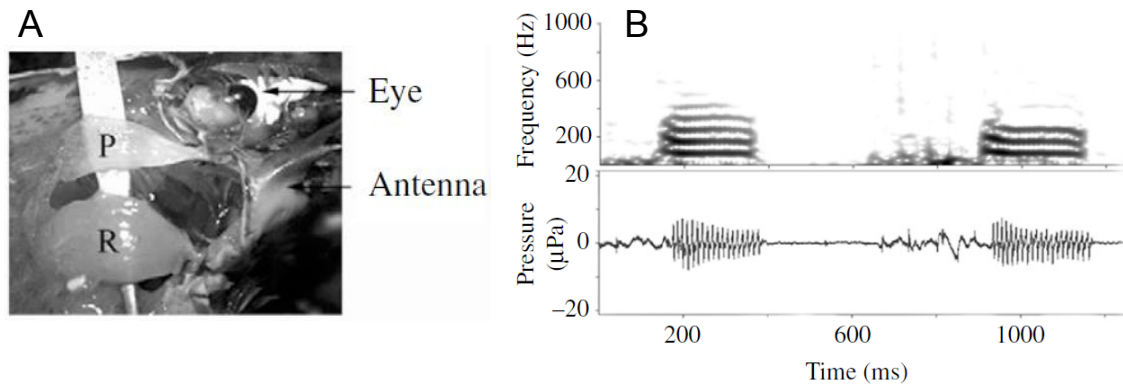


Figure 1 : A) Photographie de l'anatomie de la paire gauche de muscles (P : promoteur ; R : rémoteur) localisée à l'intérieur de la carapace et qui est responsable de la production sonore du homard Américain. En se contractant, ces muscles font vibrer la carapace qui génère un buzz basse fréquence. B) Spectrogramme (haut) et série temporelle (bas) d'un buzz. Tiré d'Henninger and Watson 2005.

Comportement territorial marqué des homards mâles

Le homard Américain a été identifié il y a près de 50 ans comme figurant sur la liste des bons modèles biologiques d'étude pour analyser des comportements complexes (Scrivener 1971, Atema and Voigt 1995). Les homards mâles présentent notamment des comportements très agressifs entre individus (Figure 2). En effet, ils utilisent des rencontres agonistiques pour établir et maintenir au sein d'un groupe une relation de dominance pour obtenir un meilleur accès aux terriers et aux femelles lors de la période de reproduction (Atema and Cobb 1980). Lorsque deux individus se rencontrent, ils effectuent un panel de comportements agonistiques allant de l'intimidation visuelle aux contacts physiques (Scrivener 1971, Huber and Kravitz 1995). Le perdant de la rencontre (i.e. le dominé) évite ensuite le gagnant (i.e. le dominant), et la dominance est maintenue via deux moyens de communication. Les signaux chimiques (i.e. phéromones) libérés dans les urines sont le meilleur moyen de conserver la mémoire du résultat entre paires d'individus après une rencontre agonistique (Breithaupt and Atema 1993, Karavanich and Atema 1998, Breithaupt et al. 1999). Les signaux visuels joueraient aussi un rôle dans la reconnaissance d'individus spécifiques (Gherardi et al. 2010, Bruce et al. 2018). Cette capacité à rappeler le résultat des rencontres passées aide les homards à éviter des combats

supplémentaires et permet ainsi de réduire les risques de blessures supplémentaires (Breithaupt et Atema 2000). Ce comportement a également été démontré chez le homard Européen (Skog 2009). Cependant, le rôle potentiel des sons comme communication intra-spécifique durant ces rencontres agonistiques, s'il existe, n'est pas encore connu (Breithaupt 2002). En fait, la littérature laisse plutôt supposer que les sons ne sont pas utilisés, certaines études rapportant qu'aucun buzz n'était enregistré durant des rencontres agonistiques effectuées en laboratoire (Scrivener 1971, Atema and Cobb 1980, Atema and Voigt 1995). Nous verrons par la suite que ce constat est dû à la propagation acoustique en cuve, et non à la production sonore des animaux.



Figure 2 : Photographie d'une rencontre agonistique entre deux homards Américains mâles. © Atema

Plan de la partie 1

En nous basant sur ce corpus de connaissances présenté dans la littérature bioacoustique et comportementale du homard Américain, nous avons effectué trois différentes études bioacoustiques complémentaires d'abord sur le homard Européen (**Chapitres 1 et 2**), et ensuite sur le homard Américain (**Chapitre 3**) :

Le **Chapitre 1** présente la première caractérisation des sons produits par le homard Européen en cuve, en tenant compte de l'effet physique des cuves sur les sons.

Le **Chapitre 2** étudie le comportement acoustique de homards Européens mâles lors de rencontres agonistiques.

Le **Chapitre 3** rapporte les capacités auditives du homard Américain dans le but de renforcer (ou réfuter) le rôle écologique des buzz pour la communication intraspécifique.

Chapitre 1 : Caractérisation sonore du homard Européen en cuve



Résumé

La première étape d'un processus expérimental visant à caractériser les sons produits par un organisme marin (de taille raisonnable) consiste à isoler cet animal dans une cuve. Ce travail est préliminaire aux expérimentations qui seront ensuite réalisées *in natura*. En effet, cela permet de s'affranchir des autres sources sonores présentes dans le milieu marin, et rend possible les observations visuelles pour associer de façon certaine un son à un comportement particulier.

Cependant, les effets physiques des cuves sur les sons et les biais qu'ils induisent doivent être pris en compte dans la caractérisation des sons. C'est un phénomène déjà largement connu dans la littérature acoustique (e.g. Stanton and Beyer 1978, Pierce 1981, Schroeder 1996), mais qui reste étonnamment peu considéré dans la littérature bioacoustique. Les plus anciens articles discutant de ce phénomène sur des sons biologiques sont issus de Parvulescu (1964, 1967), mais sont purement théoriques et ne présentent aucune forme de quantification*. Ce n'est que récemment qu'Akamatsu et al. (2002) ont illustré (théoriquement et expérimentalement) l'impact physique des cuves sur les sons biologiques. Chaque cuve possède ses propres fréquences de résonance, qui vont dépendre de sa forme, sa dimension et de son volume d'eau. Si la fréquence sonore est plus basse que la fréquence de résonance minimale, le son va être fortement atténué car sa longueur d'onde est plus grande que la taille de la cuve (e.g. pour 100 Hz, la longueur d'onde est d'environ 15 m). A l'inverse, si la fréquence sonore est proche ou au dessus de cette fréquence de résonance minimale, le son va être déformé : la série temporelle enregistrée sera plus longue que le son émis (réverbération), et son spectre comprendra des pics non présents dans le signal source (résonance). Deux nouvelles études ont ensuite souligné l'incompatibilité des cuves pour des expérimentations d'écoute chez les poissons (Duncan et al. 2016, Rogers et al. 2016). Il est important de noter que toutes les études

* Les références de Parvulescu sont citées dans la plupart des articles récents de bioacoustiques en cuve. Dans le cadre de cette thèse, il nous a fallu plusieurs semaines et l'aide d'un bibliothécaire professionnel pour les trouver. Nous avons alors découvert qu'il s'agit de la retranscription d'une discussion ayant eu lieu en conférence. Cette référence ne contient malheureusement aucune information utile pour comprendre et/ou quantifier l'impact de cuves sur les sons enregistrés.

citées ici proviennent de la littérature bioacoustique des poissons, qui produisent principalement des sons à bande de fréquence étroite (i.e. sinusoïdes).

Après la réalisation d'un état de l'art de la bioacoustique des crustacés (voir Tableau récapitulatif en Annexe 1), nous avons constaté que :

- * une grande partie de la littérature se focalise sur les différentes espèces de langouste,
- * les langoustes (et la plupart des crustacés) émettent majoritairement des impulsions à large bande de fréquence (i.e. des sons différents de ceux des poissons),
- * les effets acoustiques des cuves, pourtant particulièrement marqués sur les sons larges bandes, sont largement ignorés.

Dans ce contexte, il nous a paru important de quantifier et présenter ici ces effets. Nous avons d'abord étudié la distorsion d'un bruit blanc artificiel émis par une source sonore dans des cuves de différentes tailles. Ce bruit avait pour but de simuler un son à large bande, avec des fréquences typiques des crustacés. Le signal enregistré dans les différentes cuves était différent de l'original, avec l'apparition de fréquences pics qui diminuaient avec l'augmentation de la taille des cuves. Ces fréquences correspondaient aux fréquences de résonance minimale des cuves (Akamatsu et al. 2002). De plus, un creux d'intensité était observé sous la fréquence de résonance minimale, qui s'explique par l'atténuation des basses fréquences. En tenant compte de ces résultats, nous avons ensuite caractérisé les sons du homard Européen durant deux types de comportements : lors de l'alimentation, et en cas de stress.

Lorsqu'il s'alimente, le homard produit des trains d'impulsions large bande similaires à ceux décrits dans la littérature bioacoustique des crustacés. Ces sons sont ainsi déformés par les fréquences de résonance, et leurs caractéristiques fréquentielles et énergétiques ne peuvent pas

être mesurées en cuve. De plus, la réverbération des impulsions rallonge leur durée. Cependant, d'autres caractéristiques temporelles telles que le temps inter-impulsion peuvent être calculées.

Nous montrons également de façon pionnière que lorsqu'il est stressé, le homard Européen émet un son basse fréquence (~ 100 Hz) similaire au buzz du homard Américain. Bien qu'il ne soit pas impacté par les fréquences de résonance et la réverbération, ce son est fortement atténué, et des niveaux énergétiques estimés à un mètre des individus ne peuvent pas être calculés sans utiliser des modèles complexes de propagation.

Nous avons ainsi quantifié les effets physiques des cuves (résumés dans un tableau méthodologique dans la Discussion) sur les sons produits par les crustacés. Comprendre et quantifier ces phénomènes est critique, car les ignorer conduit à une interprétation erronée des mesures sonores et engendre le risque de passer à côté d'importantes hypothèses sur le rôle écologique des sons étudiés. Nous discutons également dans ce chapitre du rôle écologique potentiel des buzz, car malgré l'absence d'études sur leur comportement acoustique, la littérature bioacoustique des crustacés suggère qu'ils pourraient être détectés par le homard lui-même.

Article n°1:

Sound characterization of the European lobster
***Homarus gammarus* in tanks**

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Abstract

Experiments in marine behavioural ecology rely heavily on observations made in tanks. However, when studying acoustic behaviours of marine animals in confined volumes, the effects of reverberation must be characterized, something that has been overlooked in parts of the marine ecology literature. In this study, we characterized reverberation in tanks using an artificial sound source and examined the implications for bioacoustic studies using sounds emitted by the European lobster *Homarus gammarus* during feeding and in response to stress. Broadband and transient sounds commonly produced by crustaceans were severely impacted by reverberation such that their spectral characteristics and pulse width durations could not be assessed. In contrast, low frequency sounds could be characterized in tanks, but not their source level. Based on these observations, we describe a simple methodology to identify which sound characteristics can be measured in tanks. When feeding, the lobsters produced broadband and transient sounds called ‘rattles’, similar to sounds reported for tropical spiny lobsters *Palinurus longipes* and *P. argus*. When stressed, *H. gammarus* vibrated its carapace, producing a low-frequency sound analogous to the ‘buzzing’ sound of the American lobster *H. americanus*. The potential role of species-specific sound is discussed; however, although our observations represent the first bioacoustic characterization of *H. gammarus*, additional behavioural studies are necessary to understand their ecological meaning.

Key words

European lobster | Passive acoustics | Tanks | Reverberation | Rattle | Buzzing sound | Spectral analysis

Introduction

Bioacoustic studies of crustaceans have recently been receiving more attention in marine ecology (e.g. Edmonds et al. 2016). This has been driven partly by the commercial value, ubiquitous distribution and apparent ease of study of crustaceans compared with larger, highly mobile mammals. However, while the purpose of sounds emitted by whales and dolphins has been investigated for decades (Tyack and Clark 2000), little is known about the ecological roles of sounds made by crustaceans (Coquereau et al. 2016a, b, Edmonds et al. 2016). Recent studies have suggested that the sounds emitted by temperate marine decapods have properties suitable for *in situ* bio acoustic studies (e.g. *Maja brachydactyla*, Coquereau et al. 2016a).

Generally, marine crustaceans are known to produce a variety of sounds through different mechanisms, ranging from ‘stridulation’ in crabs (Guinot-Dumortier and Dumortier 1960, Boon et al. 2009) to ‘cavitation bubble collapse’ in snapping shrimps (Knowlton and Moulton 1963, Versluis et al. 2000), ‘stick and slide friction’ in palinurids (Meyer-Rochow and Penrose 1976, Patek 2001) and ‘carapace vibration’ in nephropids and stomatopods (Henninger and Watson 2005, Patek and Caldwell 2006). Most reported sounds in the bioacoustic literature on crustaceans are broadband and transient (Au and Banks 1998, Patek et al. 2009, Coquereau et al. 2016a, b). Authors have suggested many hypotheses about the roles of these sounds, including anti - predator defense in palinurids (Bouwma and Herrnkind 2009, Buscaino et al. 2011) and intraspecific communication in paddle crabs (Buscaino et al. 2015). Some sounds are described as a consequence of identifiable activities, such as the ‘rattles’ emitted while feeding in palinurids (Moulton 1957, Meyer-Rochow and Penrose 1976). A few crustacean species, including the American lobster *Homarus americanus* (Milne Edwards 1837), have been reported to emit low-frequency and narrowband sounds (Fish 1966, Henninger and Watson 2005). When threatened or handled, the carapace of *H. americanus* vibrates and leads to a ‘buzzing sound’, due to the contraction of internal muscles located at the base of the second

antenna (Fish 1966, Mendelson 1969, Henninger and Watson 2005). The European lobster *H. gammarus* (Linnaeus 1758), which is closely related to the American lobster, has a similar anatomical morphology (Holthuis 1991), but no studies have yet reported on the sounds emitted by this species.

Most of the bioacoustic studies mentioned above have been performed in tanks because this permits the visual observations necessary to associate sounds with precise behaviours (Hazlett and Winn 1962a,b, Meyer-Rochow and Penrose 1974, 1976, Mulligan and Fischer 1977, Patek and Caldwell 2006, Patek and Baio 2007, Patek et al. 2009, Buscaino et al. 2011, 2015, Coquereau et al. 2016a,b). However, the sound field in a tank is highly complex because of the interference caused by multiple reflections on the tank walls. Usually there are so many reflections that individual echoes cannot be resolved. This phenomenon is called reverberation and can be seen in data as the persistence of sound after its emission stops. It can prevent animal sounds from being properly characterized in tanks (Parvulescu 1964, 1967, Akamatsu et al. 2002). When reverberation occurs, standing waves may be generated by the superposition of reflected sound waves. Thus, what is recorded may correspond to the resonant frequencies of the standing wave because of its longer duration relative to the biological sound that was emitted (Akamatsu et al. 2002). The recorded spectrum becomes distorted and difficult to characterize. A large body of acoustic literature exists on the effects of reverberation when measuring broadband sounds (e.g. Pierce 1981, Schroeder 1996) but appears to have been largely overlooked within the bioacoustic community, although highlighted in the 1960s (Parvulescu 1964, 1967). However, the problem with reverberation has recently gained interest in this field in acoustic pressure (Akamatsu et al. 2002) and particle motion measurements (Duncan et al. 2016, Popper and Hawkins 2018).

Nevertheless, bioacoustic studies in small tanks can still provide reliable information. Recently, Akamatsu et al. (2002) described for the first time the sound distortions produced in

small tanks by combining empirical approaches with calculations from acoustic theory. This important, fundamental article focused on low-frequency and narrowband sounds emitted by fish. As reverberation is highly dependent on the frequency of interest with respect to the tank's resonant frequencies (which in turn depends on the tank's dimensions), the following applies: if the considered sound has a frequency on the order of, or higher than, the tank minimum resonant frequency, it will be affected by reverberation. The sound's duration is extended and its frequency content may be altered. On the contrary, if the considered sound has a frequency largely below the tank minimum resonant frequency, then the recorded sound is not impacted by reverberation (its duration and frequency content not being altered).

The purpose of this study was to characterize sounds produced by *H. gammarus* individuals during different activities and compare them with published information on *H. americanus* and other decapods. But, considering the limits of small tanks for bioacoustic studies, the first step was to determine the conditions under which reliable information on sounds emitted by this species can be collected.

Materials and Methods

All laboratory experiments were carried out at the Océanopolis public aquarium in Brest (Brittany, France).

The lobster *Homarus gammarus* (Crustacea, Malacostraca, Nephropidae) is a large (up to 6 kg), mobile, nocturnal and commercially important crustacean in European coastal waters (Smith et al. 1998). Its life cycle is typical for a benthic crustacean, with a pelagic larval stage followed by benthic juvenile and adult stages and growth occurring through successive periods of molts (Cobb and Wahle 1994, Sheehy et al. 1999, Agnalt et al. 2007).

Animal collection, housing and care

Seventeen *H. gammarus* individuals (10 females and 7 males) with a carapace length (CL) between 8.7 and 12 cm were collected through snorkeling in the Bay of Plougonvelin (Brittany, France) at depths between 1 and 10 m during January and February 2017. All individuals were transferred to a shaded, outdoor polyester circular tank (radius = 4 m, effective height = 1.13 m; seawater volume = 14.2 m³) for holding. The tank was continuously supplied with sand-filtered, UV-sterilized seawater pumped from the Bay of Brest. Temperature, salinity and animal conditions were controlled daily. During experiments, temperature varied between 9 and 12.5°C and salinity was between 32.8 and 34.6. Animals were fed with frozen squid, frozen mackerel and fresh mussels *ad libitum* and were kept under the natural photoperiod in this tank. Abundant sections of rigid PVC drainage pipes were provided as shelters. Lobsters were held together in the tank during the entire period of the study (around 4 mo, depending on the date of capture) when they were not being used for the experiments. No lobsters were harmed during the study. At the end of the study, all lobsters were transferred to the Océanopolis public aquarium.

Experimental tanks

Three types of rectangular tanks were used: 6 identical glass tanks ($0.60 \times 0.50 \times 0.35$ m, length \times width \times effective height; 0.105 m³), 1 plastic tank ($1.14 \times 0.92 \times 0.45$ m; 0.47 m³) and 1 larger polyester tank ($2.10 \times 2.10 \times 0.53$ m; 2.34 m³). During experiments, tanks were continuously supplied with the same seawater flow as for the holding tank. The 6 glass tanks and the plastic tank were in a slightly warmer room than the polyester tank, which resulted in somewhat warmer water conditions in the glass and plastic tanks (13.9–14.5°C) than in the polyester tank (9.0–12.5°C) during the study.

Recordings

Sounds were recorded using a pre-amplified hydrophone (HTI-92-WB, High Tech) with a sensitivity (SH) of -155 dB re 1 V μ Pa⁻¹ and a flat response from 2 to 50 kHz. The hydrophone was connected to a compact autonomous recorder (EA-SDA14, RTSys) powered by battery to limit electronic self-noise. Recordings were made with a sampling frequency (F_s) of 156 kHz at 32-bit resolution. Sounds, $S(t)$, were recorded in volts and then converted to pressure, $p(t)$ in μ Pa, in the time-domain (t), using the following equation:

$$p(t) = S(t) \times 10^{\frac{-G}{20}} \times D \times 10^{\frac{-SH}{20}} \quad \text{Eq. 1}$$

where G (dB) is the recorder gain (here $G = 14.7$ dB), D is a constant for the dynamic response of the recorder (2 V for this model) and SH is the sensitivity of the hydrophone.

Finally, to associate a sound with a particular behavioural event, both visual observations and video recordings (GoPro® HERO3 camera) were made during experiments.

Reverberation in the experimental tanks

To quantify distortion in the 3 types of experimental tanks (0.105 m³ glass tank, 0.47 m³ plastic tank and 2.34 m³ polyester tank), an artificial sound was emitted into each one with an omnidirectional underwater speaker (AQUA 30, DNH, 8 Ohms, 20–20 000 Hz) associated with an amplifier (Plug and Play 12 W) connected to a computer. During recordings in the tanks, the

water pumps were switched off to reduce the background noise to a minimum. No animals were present in the tanks during these measurements. Sound was emitted for 2.5 s, with an intensity spread equally over a wide band of frequencies (between 0 Hz and 24 kHz) to simulate white noise. The sound was emitted 5 times at different distances from the hydrophone, from 0.1 to 1.5 m. Where peak frequencies appeared in the recorded white noise and had the same power spectrum level at different distances in a particular tank, these corresponded to the tank's resonant frequencies (Akamatsu et al. 2002). Recorded peaks were then compared to the theoretical resonant frequencies ($f_{\text{rectangular}}$, Hz) of a rectangular glass tank with the dimensions L , W and H (after Akamatsu et al. 2002):

$$f_{\text{rectangular}} = \frac{c}{2} \sqrt{\left(\frac{l}{L}\right)^2 + \left(\frac{m}{W}\right)^2 + \left(\frac{n}{H}\right)^2} \quad \text{Eq. 2}$$

where c is the sound velocity in the seawater (approximated at 1500 m s⁻¹ in our case), l , m and n represent integers (≥ 1), and the combination of these is called the 'mode number'. The minimum resonant frequency is then defined at mode (1, 1, 1) for a particular tank dimension (Akamatsu et al. 2002).

Experiments

Sounds produced by individual lobsters during 2 different behaviours—feeding and response to stress—were recorded between March and May 2017 in the 8 tanks described above. During this period, 3 male lobsters molted within 1 wk of each other. We took advantage of this to compare sounds emitted between intermolt (i.e. hard shell) and postmolt (i.e. soft shell) lobsters under the same conditions.

Feeding

Rattles were emitted when lobsters were feeding. Six lobsters (3 postmolt males, 3 intermolt males) were used in each tank type, and sound recordings were made with all 3 different food types: frozen squid, frozen mackerel and fresh mussels. Sound recordings started

at least 10 min after the introduction of the hydrophone in the tanks, and food was introduced into the tank below the hydrophone. Recordings ended when all added food had been consumed; the sessions lasted from 10 min to several hours.

Response to stress

Buzzing sounds were provoked by handling organisms as described in the literature for American lobsters (Fish 1966, Henninger and Watson 2005). All 17 lobsters were tested in each tank type. Individuals were gently lifted and maintained above the bottom of the tank for 20 s to 1 min in front of the hydrophone at distances between 10 and 20 cm for each recording.

Conditions of tank recordings

The hydrophone was suspended at the center of each tank, 20 cm above the bottom. Silicone mats (0.5 cm thick) were placed on the bottom of the glass-sided tanks to prevent sounds caused by the hard body parts of lobsters striking, or moving across, the glass. The ‘daylight’ conditions for experimental tanks were simulated using fluorescent light tubes above the tanks. The top of each tank was partially covered (25% of the total surface) by a polystyrene sheet above, but not touching the water surface, to create a shaded zone.

During sound recordings, the water pumps were switched off to reduce the background noise to a minimum. The background noise in the experimental tanks was recorded prior to each recording experiment without lobsters. Spectra for the background noise were flat (around 40 dB re 1 $\mu\text{Pa}^2 \text{Hz}^{-1}$), between 50 Hz and 75 kHz, with no typical peak frequencies compared to those that were present during experimental recordings with lobsters. At frequencies below 50 Hz, peaks were present (up to 90 dB re 1 $\mu\text{Pa}^2 \text{Hz}^{-1}$) and corresponded to instrumental self-noise from the recorder. These were excluded from the biological sounds’ analysis.

Then, an individual lobster was carefully transferred to the experimental tank from the separate holding tank. Acclimatization lasted at least 2 d before recordings began, and animals continued to be fed *ad libitum* during this period. Recording sessions started after the

individuals were considered acclimatized to the presence of the hydrophone in their tanks (i.e. when attacks on the hydrophone stopped). After recordings, individuals were returned to the separate holding tank, and sound files were archived for analysis.

Sound analyses

Acoustic characteristics of recorded sounds Recordings of raw sounds (files in .wav format) and videos were analyzed simultaneously to associate particular sounds with behavioural events. Based on this file annotation, each sound type from the different recordings was extracted manually using Audacity® (Version 2.1.1; Audacity Team 2015). Then, subsampled data from the converted recordings were analyzed between 1 and 78 kHz for the identified rattle sequences and between 60 and 500 Hz for identified buzzing sound sequences. All sequences were processed using custom-made MATLAB (Version 9.1; 2016b) scripts. The following characteristics were calculated.

The sound pressure level (SPL, in dB re 1 μ Pa) was calculated for a time window equal to the length (T) of the selected sound (Erbe 2010). For characterizing broadband and transient rattles, the peak-to-peak SPL, SPL_{pp} , was calculated as:

$$SPL_{pp} = 20\log[\max(p(t)) - \min(p(t))] \quad \text{Eq. 3}$$

where $\max(p(t))$ is the maximum value and $\min(p(t))$ the minimum value for the period, T . For continuous and narrowband buzzing sounds, the root-mean square SPL, SPL_{rms} , was calculated as:

$$SPL_{rms} = 20\log\left(\sqrt{\frac{1}{T} \int_T p(t)^2 dt}\right) \quad \text{Eq. 4}$$

The power spectral density was estimated by the periodogram, γ (in dB re 1 μ Pa² Hz⁻¹):

$$\gamma(f) = |P(f)|^2 \quad \text{Eq. 5}$$

with $P(f)$ being the Fourier transformation at frequency f of the time-domain signal $p(t)$:

$$P(f) = \int_T p(t) e^{-2j\pi ft} dt \quad \text{Eq. 6}$$

The peak frequency (f_p , in Hz) is defined as the frequency at which the power spectral density is maximal. If the power spectral density contains several peaks, the frequency of the largest peak is called the first peak frequency.

Frequency bandwidth (B , Hz), was estimated as the measurement of the spread of the power spectral density (standard error) around the first peak frequency:

$$B = \frac{\sqrt{\int (f - f_p)^2 \gamma(f) df}}{\int \gamma(f) df} \quad \text{Eq. 7}$$

Finally, in addition to rattles composed of trains of pulses (Figure 3), we also calculated: (a) the duration of the entire sound ($T1$, in ms); (b) the pulse-to-pulse time interval ($T2$, in ms); (c) the total number of pulses per train (n); and (d) the pulse rate (R , in Hz), defined as the number of pulses per train (n) divided by the sound duration ($T1$). Time characteristics were calculated using the first attack of each pulse (Fig. 1).

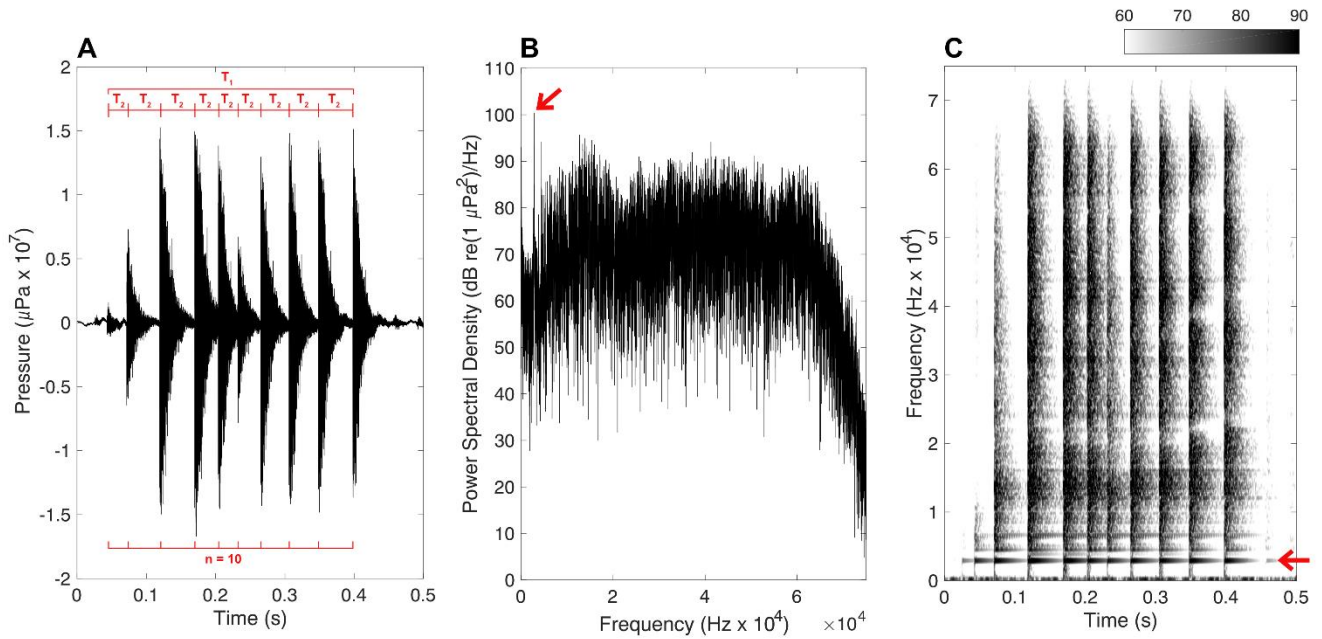


Figure 3: Example of a typical rattle emitted in a 0.105 m^3 glass tank by a European lobster (10.6 cm carapace length) plotted using 3 different time-series analyses. (A) Oscillogram, showing how the following characteristics were measured: the number of pulses per train (n), the duration of the entire sound (T_1) and the pulse-to-pulse time interval (T_2). Time characteristics were calculated using the first attack of each pulse. (B) Acoustic spectrum (FFT size: 78126). (C) Spectrogram (FFT size: 1024; Hamming window: 501 points; 99% overlap). The red arrow indicates the first peak frequency (2.8 kHz) and corresponded to the minimum resonant frequency computed for the 0.105 m^3 tank (2.9 kHz). The color scale is in $\text{dB re } 1 \mu\text{Pa}^2 \text{ Hz}^{-1}$.

Results

Distortion of a known sound through tank reverberation

From a white noise emitted, i.e. with the power spectral density being constant over a wide band of frequencies, signals recorded in different types of tanks were not flat and showed several peaks (Table 1). The first peak frequencies decreased as the dimensions of the tanks increased, ranging from 2.8 kHz for a 0.105 m³ tank to 1.8 kHz for the 2.34 m³ tank. These peaks occurred at frequency values matching the minimum theoretical resonant frequency calculated in the 0.105 m³ tank at 10 and 32 cm from the hydrophone (2.8 and 2.9 kHz, respectively). The same result was found for the 0.47 m³ tank at 10 cm from the hydrophone (2 kHz), while peaks at 2.7 kHz at 42 and 72 cm were also found, corresponding to another resonant frequency calculated with the mode (2, 2, 1), even if the peak at 2 kHz was still present. For the 2.34 m³ tank, the first frequency peak was 1.8 kHz for the 3 distances from the hydrophone, and corresponded to a resonant frequency of mode (1, 1, 2); a peak at 1.5 kHz (the minimum resonant frequency of this tank) was also present. The power spectral density of the recorded white noise showed several other peak frequencies up to the minimum resonant frequency in each tank (until almost 20 kHz).

Table 1: Sound characteristics calculated for artificial white noise emitted at different distances from the source and in different tanks used for sound recording experiments. Results are presented as mean (\pm SD). No animals were present during these recordings. *f_p*: peak frequency; *f_{rectangular}*: theoretical resonant frequency.

Tank size (m ³)	0.105		0.47			2.34		
Distance from the hydrophone (cm)	10	32	10	42	72	10	80	150
<i>f_p</i> (kHz)	2.8 (0)	2.8 (0)	2 (0)	2.7 (0)	2.7 (0)	1.8 (0)	1.8 (0)	1.8 (0)
<i>f_{rectangular}</i> (kHz)	2.9	2.9	2	2	2	1.5	1.5	1.5

Figure 3 shows a rattle emitted by an intermolt male lobster (10.6 cm in CL) during feeding experiments in a 0.105 m³ tank. The first peak frequency of this broadband sound was 2.8 kHz (red arrow in Figure 1), as also found for the recorded white noise in the same tank. It corresponded to the minimum resonant frequency of this tank, showing a net distortion of the sound.

Taking into account these results, we therefore calculated only time characteristics for the rattles, and SPL and spectral characteristics for buzzing sounds.

Recordings made during feeding

Rattles were defined as sound bursts consisting of a pulse train over a broadband spectrum (Figure 3). A total of 168 rattles were recorded during feeding experiments with the six male lobsters (Table 2). These occurred when animals were feeding on all three types of foods. For almost one month after molting, we did not record any rattles from the three soft lobsters, regardless of food type consumed. The time characteristics of these sounds were highly variable (Table 2): *T*₁ varied between 44 and 960 ms (mean ± SD, 223.6 ± 145.2 ms), *T*₂ varied between 1 and 89 ms (65.1 ± 13.8 ms), *n* varied between 3 and 41 pulses per train (12 ± 7.8) and *R* varied between 6.11 and 200 Hz (65.1 ± 39.2 Hz).

Table 2: Sound characteristics calculated in the European lobster rattles and buzzing sounds. The results are presented as means (±SE). I: interference due to tank reverberation; NA: not applicable. T1: duration of the entire sound; T2: pulse-to-pulse time interval; n: total number of pulses per train; R: pulse rate; SPL_{pp}: peak-to-peak sound pressure level; SPL_{rms}: root-mean square sound pressure level.

	No. of ind.	T ₁ (ms)	T ₂ (ms)	<i>n</i>	R (Hz)	SPL (dB re 1 μPa)	First peak frequency	Second peak frequency	Band width
Rattles	6	223.6 (145.2)	12.4 (13.8)	12 (7.8)	65.1 (39.2)	SPL _{pp} I	kHz I	kHz I	kHz I
Buzzing sounds	7	230 (187.5)	NA	NA	NA	SPL _{rms} 106.4 (6.1)	Hz 100.9 (19.6)	Hz 201.8 (39.2)	Hz 56.1 (22.5)

Carapace vibration in a response to stress

Buzzing sounds were defined as continuous sounds with a narrowband spectrum. A total of 189 buzzing sounds were recorded as a stress response by seven individuals (one intermolt female, three intermolt males and three postmolt males, ranging from 9 to 12 cm in CL) of the 17 lobsters tested (Table 2). These sounds occurred in association with carapace vibrations (felt by hand) and were often associated with tail flips. Buzzing sounds were also recorded from the two most aggressive intermolt male lobsters (10.6 and 12 cm in CL) just before they attacked the hydrophone in the 0.105 m³ tanks during feeding experiments. These sounds had a mean first peak frequency of 100.9 Hz (range 66.6 to 152.6 Hz) and were mainly (92% of the analyzed recordings) accompanied by a second, strong harmonic with a mean frequency at second peak intensity of 201.8 Hz (range 123.2 to 305.2 Hz; Table 2). The number of harmonics detected varied from 2 to > 10 for the most intense buzzing sounds. The buzzing sounds tended to decrease in frequency over time (Figure 4), showing a net, mean frequency modulation of 56.1 Hz when the onset dominant frequency was compared to the dominant frequency at the end of the sound. The harmonics showed the same pattern. Overall, the buzzing sounds had a mean SPL of 106.4 dB re 1 µPa rms (range 92.1 –119.3 dB re 1 µPa rms), and could be as long as 1600 ms (mean = 230 ± 187.5 ms; Table 2).

The three soft lobsters tested during the feeding experiments were also observed to be capable of emitting buzzing sounds, and more frequently than the intermolt lobsters. We observed no significant differences in buzzing sounds emitted between postmolt and intermolt lobsters in the sound duration, SPL and peak frequencies (MW, $p > 0.05$). However, the bandwidth was significantly larger for intermolt lobsters compared to postmolt lobsters (MW, $p < 0.05$). In addition, one postmolt lobster (12 cm CL) emitted 3 buzzing sounds with the highest SPL (> 119 dB re 1 µPa rms) values observed here.

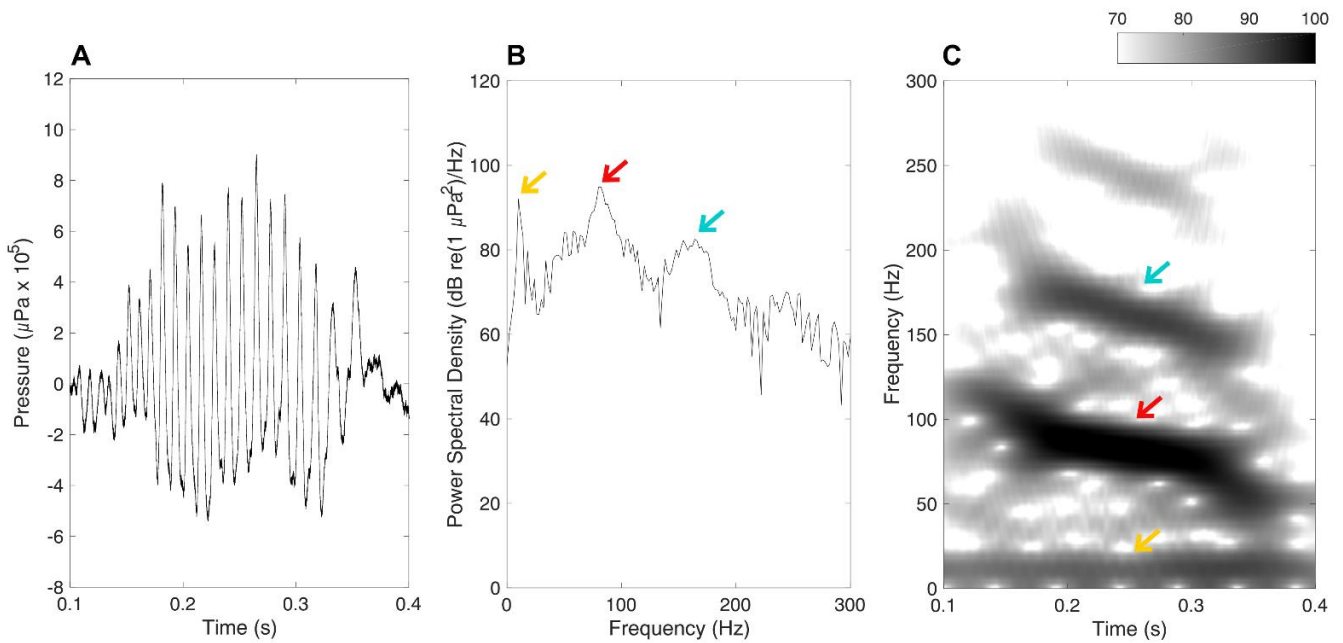


Figure 4: Example of a buzzing sound produced in a 0.105 m³ glass tank by a ‘soft’ (postmolt) European lobster (9.5 cm carapace length) plotted using 3 different time-series analyses. (A) Oscillogram; (B) acoustic spectrum (FFT size: 46876); (C) spectrogram (FFT size: 2048; Hamming window: 501 points; 99% overlap). The yellow arrow indicates instrumental self-noise from the recorder, the red arrow indicates first peak frequency and the blue arrow indicates the second harmonic. The color scale is in dB re 1 $\mu\text{Pa}^2 \text{Hz}^{-1}$.

Discussion

Quantification of bioacoustic signatures in tanks

Characterizing reverberation in the different tanks required using known sound in order to be able to quantify how the tank geometry distorted sounds. As expected, reverberation distorted the white noise used in our tests for all the tanks. Several peak frequencies appeared corresponding to the calculated theoretical resonant frequencies of the different tanks (Table 1). The same result was also found for recordings of broadband rattles emitted by the lobsters in these tanks (Figure 3). Based on these observations, and inspired by Akamatsu et al. (2002), we summarized these results as a guideline for characterizing marine crustaceans' sounds in small tanks (Table 3).

Although we recognize that this information exists in various forms in the acoustic literature, it seems important to present these guidelines directly to the bioacoustic community, where studies of crustaceans in tanks are frequently described in the literature (Hazlett and Winn 1962a,b, Meyer-Rochow and Penrose 1974, 1976, Mulligan and Fischer 1977, Patek and Caldwell 2006, Patek and Baio 2007, Patek et al. 2009, Buscaino et al. 2011, 2015, Coquereau et al. 2016a, 2016b). Most of these studies did not attempt to quantify reverberation effects on the broadband and transient sounds. For example, Meyer-Rochow and Penrose (1976) found that spectral characteristics of the squeak produced by the rock lobster *Palinurus longipes* were strongly influenced by the size and material of their recording tanks, and finally concluded that the bandwidth was uniform over the audio range. Different types of sound characteristics are commonly presented in the bioacoustic literature, including: spectral characteristics (peak frequencies, bandwidth), time (duration, pulse rate, time interpulse) and intensity levels (SPL) and source levels (SL; i.e. SPL estimated at one distance from a source). In our study, we have shown that spectral characteristics in transient and broadband sounds (such as rattles), which are usually produced by crustaceans, cannot be calculated in tanks.

Table 3: Acoustic methodology showing which sound characteristics (spectral, time, energy) can be calculated in small tanks (example: 0.105 m³) depending on the type of recorded sound. The sound characteristics were calculated selecting the entire sound. f_{res} : minimum resonant frequency of the tank; SPL: sound pressure level; SL: source energy (i.e. SPL estimated at one distance from a source)

Sound characteristics	Sound frequency	
	$< f_{res}$	$> f_{res}$
Spectral shape	Ok	No
Time (duration)	Ok	No
Time (beginning of the sound)	Ok	Ok (if sound is separated enough from other sound)
SPL	Ok	Possible if $f \gg f_{res}$
SL	No	Possible if $f \gg f_{res}$
Examples from this study	Buzzing sounds (narrowband and continuous sounds)	Rattles (broadband and transient sounds)

In contrast, low-frequency sounds (such as buzzing sounds) can be measured in tanks. Yet other analytical methods based on SPL, such as SL estimated at 1 m from a source, do not appear reliable because of the sound propagation models in use. As a reminder, Rogers et al. (2016) investigated transmission loss in the same range as our buzzing sounds in very small (0.03 m³) tanks. They showed an exponential decrease of 35 dB between 10 and 30 cm from the hydrophone, whereas based on the spherical spreading propagation model commonly used, the expected transmission loss would be estimated at only 10 dB. These results suggest that the SL values would also be unreliable in our experiments (Table 3). One solution to infer crustacean SLs in tank experiments would be to develop new propagation models, such as the one proposed by Rogers et al. (2016). This would require very accurate estimations of the source position, which is unfortunately not possible at this time for crustaceans because they are allowed to move freely in the tanks and thus cannot be considered as a fixed-point source.

Comparison with the bioacoustic literature

Rattles emitted by *Homarus gammarus* during feeding were defined as trains of broadband pulses. In the present study, first peak frequency, bandwidth and SPL were not assessed because the recordings were highly distorted due to reverberation (Table 1, Figure 3). Other authors have suggested that broadband and transient sounds emitted by marine arthropods can be characterized by sampling only the initial part of the signal (e.g. an interval less than 0.2 ms), which would correspond to a 1-way emission just before reverberation starts (Coquereau et al. 2016a). This method was not applied in the present study, because it would not determine whether the frequencies of transient sounds vary over time. For example, beaked whales emit sounds with very fast frequency modulations (Baumann-Pickering et al. 2013), which this type of signal sub-sampling would not account for. In addition, calculating a power spectral density with too few points may not fulfill the conditions for accurately estimating spectral characteristics. Thus, we preferred to focus on descriptors not impacted by tank reverberation, such as time characteristics: the duration of the entire sound, pulse-to-pulse time interval, number of pulses per train, and pulse rate.

The time characteristics of the lobsters' rattles were consistent with the feeding sounds previously reported for tropical spiny lobsters. The mean entire duration of 223 ms was of the same order of magnitude (Moulton 1957: 250 ms for *Panulirus argus*; Meyer-Rochow and Penrose 1976: 153 ms for *P. longipes*). Other types of feeding sounds recorded in temperate crustaceans had longer durations (Coquereau et al. 2016a: approximately 600 ms for *Maja brachydactyla*; Coquereau et al. 2016b: approximately 400 ms for *Cancer pagurus*). The number of pulses per signal was calculated as (mean \pm SD) 12 ± 7.8 on average for lobster rattles, which compares favourably with the reported 5 to 10 pulses per signal for spiny lobsters (Moulton 1957 for *P. argus*; Meyer-Rochow and Penrose 1976 for *P. longipes*), but is smaller than the mean of 29 ± 11 pulses per signal reported for *M. brachydactyla* (Coquereau et al.

2016a). In a general way, time characteristics in lobsters' rattles were highly variable. These rattles may be produced by the friction between mouthparts (called 'mandible grinding' in Meyer-Rochow and Penrose 1976), suggesting unintentional sounds are produced and may thus account for some of this reported variability.

Similar to the American lobster, the European lobster also produces a buzzing sound (Henninger and Watson 2005) that could be recorded by the hydrophone (Figure 4). First peak frequencies in the European lobster were within the range of reported first peak frequencies by Fish (1966; 100 to 130 Hz) and Henninger and Watson (2005; 87 to 261 Hz) for the American lobster. Sound durations, however, were highly variable (50 to 1600 ms), but were in the same range as those reported by both Fish (1966; between 100 and 500 ms) and Henninger and Watson (2005; between 68 to 1720 ms). In addition, the mean SPL was estimated to be about 10 dB re 1 μ Pa rms lower than for American lobsters (between 116.5 and 118.5 dB re 1 μ Pa rms; Fish 1966, Henninger and Watson 2005, Ward et al. 2011). A second strong harmonic was also observed in the European lobster that has not been described for the American lobster. The bandwidth showed how the buzzing sound decreased in frequency over time, suggesting that these types of modulations may be a characteristic of this sound. Similar results have indeed already been reported from stomatopods or mantis shrimp *Hemisquilla californiensis*, which generate tonal, low frequency sounds called 'rumbles' (Patek and Caldwell 2006). These same authors also observed that the first peak frequencies of the rumbles were accompanied with a second strong harmonic. Staaterman et al. (2011) reported 'rumbles' recorded in the field close to our results for lobsters buzzing sounds (an average dominant frequency of 167 Hz and a mean duration of 200 ms). Apart from these 3 marine crustacean species, a number of other marine organisms also generate similar low frequency sounds, including whales and fish (Clark and Johnson 1984, Connaughton 2004, Maruska and Mensinger 2009).

Is there a role for buzzing sounds emitted by the European lobster?

Because of the lack of field observations in the ecological context in which buzzing sounds are generated, it is only possible to speculate about their role based on comparisons with other organisms. As lobsters produced vibrations when handled (this was already demonstrated in Henninger and Watson 2005), it has been suggested that buzzing sounds may serve to deter potential predators. These sounds have a narrow bandwidth, suggesting that only organisms capable of perceiving sounds in the same band of frequencies might be targeted. Ward et al. (2011) showed that American lobsters vibrated when approached by 2 species of fish in a circular tank (cod and striped bass). Interestingly, the sound sensitivity of cephalopods, including octopuses, seems to be limited to low frequencies (100–200 Hz; Williamson 1988, Packard et al. 1990, Mooney et al. 2010). As the octopus is a well-known predator of European lobsters (Barshaw et al. 2003), these buzzing sounds may indeed be meant as a deterrence.

Buzzing sounds were also recorded just before 2 male lobsters attacked the hydrophone during feeding experiments. Lobsters are known to be territorial species living in burrows similar to those of mantis shrimp (Dingle and Caldwell 1969), and buzzing sounds might help to send signals of their presence to conspecifics in addition to chemical cues (Skog et al. 2009), to maintain territory. This type of behaviour was previously described in mantis shrimp (Patek and Caldwell 2006). Staaterman et al. (2011) recorded multiple rumbles of mantis shrimp in the field, which they termed ‘chorusing’. These rhythmic series, called ‘rumble groups’, may even constitute a type of conspecific communication.

Crustaceans, including lobsters, lack gas-filled organs (i.e. swim bladders) required for pressure detection, but may be still capable of detecting low frequency acoustic stimuli arising from particle motion (Popper et al. 2001, Edmonds et al. 2016, Popper and Hawkins 2018), such as the buzzing sounds. A large diversity of sensory receptors has been described in crustaceans, including statocysts and sensory hairs (Popper et al. 2001, Radford et al. 2016).

Two types of putative acoustic receptors on *H. gammarus* — hairfan and hair-peg organs — have been implicated in low-frequency sound and water-current detection (Laverack 1962, 1963). In addition, one study focused on sound detection by *H. americanus* showed that they are capable of detecting sounds, with the most sensitive responses at the lower frequencies (between 18.7 and 150 Hz; Offutt 1970). Our results also showed that ‘soft’ (postmolt) lobsters were able to produce buzzing sounds with the same characteristics (except for their bandwidth) as ‘hard’ (intermolt) lobsters. This suggests that the buzzing sounds may be a means of communication. New studies are needed to clearly examine the physiological and behavioural responses of European lobsters to such sounds.

Henninger and Watson (2005) showed that only 7.5% of their American lobsters (from a total of 1723 individuals tested) vibrated when handled, despite the fact that all lobsters have the anatomical capacity to produce these sounds. Their year-long survey demonstrated that all size classes of American lobster can produce buzzing sounds, with a similar distribution for both males and females. In the present study, we observed that only 7 out of the 17 lobsters tested vibrated and produced buzzing sounds when handled. We recorded buzzing sounds in 6 male and only 1 female lobster, meaning that 1 male and 9 female lobsters did not vibrate. Due to the low number of individuals tested, and to the stress of captivity, which could habituate lobsters to being disturbed, we cannot yet explain this difference in sound production between individuals. Further, we do not know at which stage of their life cycle the mechanism of sound production becomes operational and biologically useful in these animals.

Conclusions

Passive acoustic studies of marine crustaceans would clearly benefit from field measurements. Indeed, we emphasize that field studies are required to confirm the acoustic findings presented in this study and to test hypotheses about sound transmission and detection in natural soundscape. Preliminary characterization of European lobsters' buzzing sounds, however, suggests these could be difficult to record because they may be masked by other sources of low-frequency sounds, such as sea surface agitation related to wind speed (Wenz 1962) and anthropogenic noise (Clark et al. 2009). In conclusion, acoustic measurements in carefully controlled laboratory conditions together with behavioural observations remain an essential first step, and they should serve as a basis of comparison for any subsequent *in situ* research and monitoring projects.

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Chapitre 2 : Comportement acoustique des homards Européens mâles



Photographie d'une rencontre agonistique de deux homard mâles européens. Des petits accéléromètres étaient collés sur leur carapace pour enregistrer les vibrations qui produisent les buzz. Deux hydrophones (dont un est visible en haut de l'image) étaient également positionnés dans la cuve pour enregistrer les buzz produits par les homards.

Résumé

Nous avons exposé dans le Chapitre 1 comment le homard Européen produit des buzz lorsqu'il est stressé. Bien que cette production sonore chez le homard Américain soit connue depuis plus de 60 ans (Moulton 1957, Fish 1966, Mendelson 1969), son éventuel rôle dans la communication intra-spécifique n'est toujours pas identifié. Seul Ward et al. (2011) a démontré que ces buzz permettent de faire fuir des poissons prédateurs, mettant ainsi en lumière un rôle interspécifique de cette production sonore. Une lecture exhaustive de la littérature scientifique centrée sur le comportement du homard Américain lors de rencontres agonistiques entre individus mâles fait apparaître trois études qui ont tentées d'enregistrer ces buzz à l'aide d'hydrophones durant ces comportements. Cependant, dans chacune de ces trois études, les tentatives d'écoute se sont révélées infructueuses. Les auteurs ont conclu que ces buzz n'étaient pas utilisés par les homards lors de ces rencontres agonistiques, et leur rôle dans le processus de communication intraspécifique est alors resté hypothétique (Scrivener 1971, Atema and Cobb 1980, Atema and Voigt 1995).

En tenant compte de la forte atténuation des sons basses fréquences (comme les buzz) en cuve présentée dans le chapitre 1, nous avons effectué des rencontres agonistiques entre homards Européens mâles, telles qu'elles étaient décrites dans la littérature scientifique chez le homard Américain. Nous avons tout d'abord utilisé deux hydrophones placés dans chaque moitié de la cuve pour maximiser les chances d'enregistrer tous les buzz produits par les homards. Lors de cette première série d'expérimentations, nous n'avons enregistré que très peu de buzz, confirmant les résultats précédemment acquis et présentés dans la littérature. Afin de valider ces résultats, nous avons ensuite décidé de refaire ces rencontres en ajoutant au protocole expérimental de petits accéléromètres collés sur la carapace (i.e. céphalothorax) des homards. Ces accéléromètres avaient la capacité d'enregistrer les vibrations des carapaces des homards lorsqu'ils produisent les buzz (i.e. la source sonore ; Henninger and Watson 2005).

Nous avons alors montré que les homards produisaient de nombreuses vibrations de leurs carapaces lors des rencontres agonistiques, et que seulement 15 % des sons associés à ces vibrations étaient enregistrés par les hydrophones. Ce faible pourcentage s'explique par la forte atténuation des sons basses fréquences par la cuve elle-même (Jézéquel et al. 2018). Ce phénomène est également mis en évidence dans le Chapitre 4 de ce manuscrit.

Nous avons ensuite logiquement utilisé les séquences de vibrations mesurées par accélérométrie et produites par les homards dominants et dominés comme proxy des buzz pour étudier leur comportement acoustique. La majorité des buzz était produite par tous les individus à partir de la fin de la première rencontre agonistique, c'est-à-dire quand les statuts de dominance étaient déterminés à l'issue de la première rencontre. Nous avons notamment montré que les homards dominés produisent plus de buzz que les dominants. De plus, aucun buzz n'a été détecté (ni par les hydrophones, ni par les accéléromètres) lorsque les animaux étaient isolés dans chaque moitié de la cuve, avant la mise en contact des individus. Cependant les tests statistiques n'ont pas permis de valider la présence d'une communication chez les homards durant ces rencontres agonistiques. Ce résultat, largement discuté, pourrait être lié à la forte atténuation des buzz en cuve (i.e. les homards, comme les hydrophones, ne peuvent pas détecter les buzz), ou à une limitation intrinsèque à la méthode statistique utilisée.

Nous concluons *in fine* que le rôle écologique des sons émis (buzz) par les homards lors des rencontres agonistiques entre individus mâles a été précédemment sous estimé dans la littérature. En effet, notre étude a démontré la récurrente production de buzz par les homards durant ces rencontres agonistiques, relançant l'hypothèse d'une possible communication intraspécifique sonore chez cette espèce. Cette hypothèse reste toujours à confirmer car nous ne connaissons pas les capacités sensorielles des homards vis-à-vis de ces sons.

Article n°2:

**Acoustic behaviour of male European lobsters
(*Homarus gammarus*) during agonistic encounters**

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Abstract

Previous studies have demonstrated that male European lobsters (*Homarus gammarus*) use chemical and visual signals as a means of intraspecific communication during agonistic encounters. In this study, we show that they also produce buzzing sounds during these encounters. This result was missed in earlier studies because low-frequency buzzing sounds are highly attenuated in tanks, and are thus difficult to detect with hydrophones. To address this issue, we designed a behavioural tank experiment using hydrophones, with accelerometers placed on the lobsters to directly detect their carapace vibrations (i.e. the sources of the buzzing sounds). While we found that both dominant and submissive individuals produced carapace vibrations during every agonistic encounter, very few of the associated buzzing sounds (15%) were recorded by the hydrophones. This difference is explained by their high attenuation in tanks. We then used the method of algorithmic complexity to analyse the carapace vibration sequences as call-and-response signals between dominant and submissive individuals. Even though some intriguing patterns appeared for closely size-matched pairs (<5 mm carapace length difference), the results of the analysis did not permit us to infer that the processes underlying these sequences could be differentiated from random ones. Thus, such results prevented any conclusions about acoustic communication. This concurs with both the high attenuation of the buzzing sounds during the experiments and the poor understanding of acoustic perception by lobsters. New approaches that circumvent tank acoustic issues are now required to validate the existence of acoustic communication in lobsters.

Key words

Passive acoustics | Accelerometer | Buzzing sound | Carapace vibration | Tank | Acoustic communication | Dominance | Sound attenuation

Introduction

Sounds can be used by marine organisms to convey information. Numerous studies have demonstrated that marine mammals and fish use sounds to navigate, find food, communicate with conspecifics or even deter predators (e.g. Tyack and Clark 2000, Ladich 2015). By comparison, the potential role(s) of sounds amongst marine invertebrates is poorly described (Taylor and Patek 2010, Edmonds et al. 2016).

For instance, only a few crustacean species have been shown to produce sounds during behavioural interactions. The tropical spiny lobster (*Panulirus argus*) produces antennal rasps when attacked by predators (Bouwma and Herrnkind, 2009). Mantis shrimp (*Hemisquilla californiensis*) rumble to maintain their territories against conspecifics (Patek and Caldwell 2006, Staaterman et al. 2011). Semi-terrestrial crabs (the Ocypodidae) are known to produce stridulations that attract females to their burrows for mating (Popper et al. 2001). Other crustacean species have also been shown to produce sounds, but the lack of relevant behavioural studies does not yet permit validation of potential ecological roles for these sounds. In the temperate coastal waters of Brittany (France), several crustacean species produce a large diversity of sounds, but their ecological roles, if any, are unknown (Jézéquel et al. 2018, 2019).

Lobsters, particularly the American lobster (*Homarus americanus*), have been identified as a good study model for analyzing complex behaviours (Scrivener 1971, Atema and Voigt 1995, Huber and Kravitz 1995). Male *H. americanus* lobsters exhibit highly aggressive behaviours towards each other. Indeed, they use agonistic encounters to establish and maintain their dominance within a group to gain better access to shelters and females for reproduction (Scrivener 1971, Atema and Cobb 1980, Atema and Voigt 1995). When two individuals meet, they exhibit an array of agonistic behaviours ranging from visual displays to physical contact (Scrivener 1971, Huber and Kravitz 1995, Breithaupt and Atema 2000). The main factor influencing the outcome of an agonistic encounter is body size: larger individuals have a greater

chance of winning an encounter (Scrivener 1971). This results in shorter behavioural sequences compared with those for size-matched lobsters where their aggressive behaviours lead to highly stressful conditions (Atema and Voigt 1995). The loser of an encounter avoids the winner afterwards, and dominance is maintained through a variety of signals. Chemical signals (i.e. pheromones) released in urine appear to be the main means of preserving the memory of the outcome between pairs of individuals, post-encounter (Breithaupt and Atema 1993, Karavanich and Atema 1998, Breithaupt et al. 1999). Recently, Gherardi et al. (2010) and Bruce et al. (2018) showed that visual recognition of specific individuals also plays a role. The ability to recall the outcome of past encounters may help individual lobsters to avoid additional fights and lower their future risk of injury (Breithaupt and Atema 2000).

A recent study has shown that, similar to *H. americanus*, the European lobster (*Homarus gammarus*) also emits buzzing sounds when stressed (Jézéquel et al. 2018). These sounds are produced through the rapid contraction of internal muscles located at the base of their second antennae, which causes the carapace to vibrate (Mendelson 1969). These ‘buzzing’ sounds are characterized by low frequencies (~100 Hz) and have a relatively long duration (~ 200 ms; Henninger and Watson 2005, Jézéquel et al. 2018). Ward et al. (2011) suggested that *H. americanus* may only use these sounds to deter predators. Interestingly, earlier studies indicated that few buzzing sounds were produced during agonistic encounters in male *H. americanus* and it was then concluded that these sounds do not have a role for intraspecific interactions (Scrivener 1971, Atema and Voigt 1995, Atema and Cobb 1980). Hence, no study has examined the ecological role of these buzzing sounds and only one has described the behavioural patterns in *H. gammarus* during agonistic encounters (Skog et al. 2009).

The primary aim of the present study was to: (1) test whether male *H. gammarus* emit buzzing sounds during agonistic encounters, and (2) test the potential role of these sounds as acoustic communication between lobsters. For this purpose, we designed an experimental

laboratory set-up whose main feature was a tank containing the lobsters under study. The set-up also combined hydrophones to record their buzzing sounds in the tank, accelerometers on the lobsters to record their carapace vibrations (i.e. the source of the buzzing sounds) and cameras to record animal behaviour. Firstly, we developed a detailed ethogram based on the video recordings of the encounters. Secondly, we analyzed the behavioural sequences between dominant and submissive individuals. Lastly, we examined whether the sequences of buzzing sounds produced by two individuals depended on their relative size differences. We then analyzed these as call-and-response signals to explore their potential role for the communication of dominance.

Materials and Methods

All laboratory experiments were carried out at the research facilities of the Océanopolis public aquarium located in Brest (France).

Animal collection, characteristics and care

For these experiments, a total of 24 *H. gammarus* (Linnaeus 1758) male individuals, with carapace length (CL; measured from the eye socket to the posterior carapace margin for lobsters) between 8.7 and 13 cm, were used. They were collected carefully by hand while snorkeling in the Bay of Plougonvelin (Brittany, France) at water depths of between 1 and 10 m. Two samplings were done in May and July 2018; 12 individuals were collected during each session. Only intermoult individuals (following the description in Aiken, 1973) with full sets of undamaged appendages were collected and used for this study.

After capture, lobsters were separated randomly into two groups of 6 individuals each, and then transferred to different holding tanks. One group was held in a large shaded, polyester circular tank (radius 4 m, effective height 1.13 m, seawater volume 14.2 m³). The second group was held in two identical plastic rectangular tanks (1.50 m × 1.00 m × 0.5 m length × width × effective height; seawater volume 0.75 m³) with 3 individuals per tank, separated by plastic dividers. In the communal tanks, the lobsters' claws were bound with numbered rubber bands to avoid injury. These also identified each individual lobster. All holding tanks were continuously supplied with sand-filtered, UV-sterilized seawater pumped from the Bay of Brest. Temperature, salinity and animal condition were controlled twice a day. During holding, temperature varied between (mean ± s.d.) 14.8 ± 1°C (in May and June) and 17.5 ± 0.5°C (in July and August) and salinity between 34.4 ± 0.3 and 34.9 ± 0.1. Animals were fed with fresh pieces of fish (mackerel) and cephalopod (squid) ad libitum. They were kept under the natural photoperiod in the large circular tank, and under a 12 h : 12 h photoperiod in the smaller tanks, the daylight condition being simulated by fluorescent light tubes above the tanks. Sections of

rigid PVC drainage pipes were provided in abundance as shelters. Animals were acclimatized for at least 1 month in these conditions before they were used in the experiments.

Experimental set-up

All experiments were done in a dedicated plastic tank (1.13 m × 0.73 m × 0.5 m; 0.4 m³) placed in a quiet room, isolated from the main activities of the aquarium facilities (Figure 5). The bottom was covered with a thin layer of sand, 5 cm deep, to provide a foothold for the animals. Two LED light strips (B0187LXUS2, colour temperature 4500 K) were placed 50 cm above the tank to ensure good visibility for video recording by the cameras. The experimental tank was divided into two equal volumes by a removable, opaque, Plexiglas divider (6 mm thick) installed in the middle of the tank prior to introducing the animals (Scrivener 1971, Huber and Kravitz 1995, Skog et al. 2009). To do this, plastic gutters were epoxy glued on the vertical sides and along the bottom of the tank. This permitted the divider to easily slide up at the start of each experiment. The edges of these gutters were silicone sealed to eliminate any water exchange while the divider was in place. The barrier prevented the exchange of chemosensory and visual cues between the two lobster opponents before the agonistic encounter was started by removal of the divider.

Data recording

Buzzing sounds: hydrophones

Sounds were recorded using two pre-amplified hydrophones (HTI-92-WB, High Tech Inc., Long Beach, MS, USA), with a sensitivity of -155 dB re. $1 \text{ V } \mu\text{Pa}^{-1}$ and a flat response between 2 Hz and 50 kHz. Hydrophones were connected to a compact autonomous recorder (EA-SDA14, RTSys, Caudan, France) with a gain of 14.7 dB, and were powered by battery to limit electronic self-noise. Recordings were made with a sampling frequency of 156 kHz at 32-bit resolution. Even though buzzing sounds are characterized by low frequencies (~ 100 Hz;

Jézéquel et al. 2018), we chose a high sampling frequency because we wanted to cover a large frequency band, in case the lobsters produced new sounds during the experiments.

We used two hydrophones in the experimental tank to ensure most of the buzzing sounds emitted by individuals during the agonistic encounters could be recorded. One was placed in each compartment of the tank, 30 cm above the substrate, and they were separated by 55 cm from each other (Figure 5). Based on our earlier work to determine the acclimation state of the animals used (Jézéquel et al. 2018), this installation did not perturb the individuals.

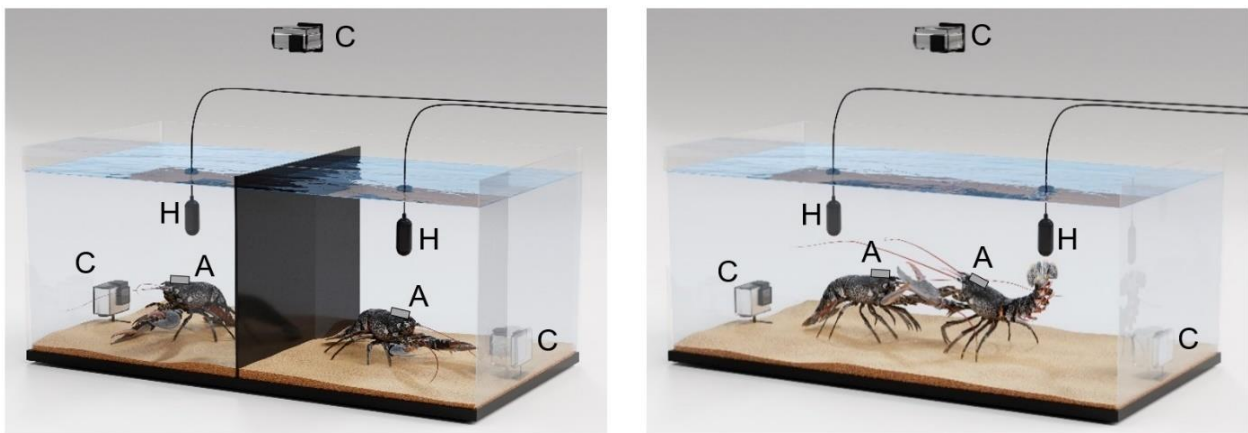


Figure 5: Experimental set-up of the agonistic encounters performed with male European lobsters, *Homarus gammarus*. Individuals were first isolated for a period of 15 min on either side of the experimental tank (left), and then the divider was lifted and we recorded agonistic encounters for another 15 min. For these experiments, we used several recording devices: two hydrophones (H), two accelerometers (A) and three cameras (C).

Carapace vibrations: accelerometers

During preliminary trials, we noted that very few buzzing sounds were recorded by the hydrophones during agonistic encounters. Lobsters emit buzzing sounds through rapid contractions of internal muscles located at the base of their antennae, which vibrate the carapace (Henninger and Watson 2005); we therefore added accelerometers on their carapaces as a means to detect carapace vibration events, independently of the hydrophones.

One small AX-3 data logger (23 × 32.5 × 8.9 mm, mass 11 g; Axivity Ltd, Newcastle Helix, UK) was glued with 3 min underwater epoxy to the dorsal carapace of each lobster, near

the eye sockets at the base of the second antennae (Figure 5). The x-axis was oriented parallel to the longitudinal body axis, which is also parallel to the internal muscles responsible for the carapace vibration (Henninger and Watson 2005). The accelerometers were set to record acceleration in all three axes (range ± 16 g, 156.96 m s^{-2}) with a sampling frequency of 3200 Hz and a 13-bit resolution. The accelerometers had a 512 MB memory card onboard. Each accelerometer was waterproofed before attachment by encasing it in a polyethylene film sealed shut with heat-shrink tape. Air trapped inside the polyethylene film made the accelerometer loggers neutrally buoyant in seawater. All accelerometers were marked with unique numbers to associate them with particular individuals. This technique permitted us to link each carapace vibration recorded to an individual and also to validate the buzzing sounds recorded with the hydrophone recordings. As stated above for the hydrophones, we did not observe any evidence that the presence of the sensors perturbed their movements during the experiments.

Movements: video

Visual observations and video recordings were made during all experiments using three GoPro® HERO3 cameras. Two cameras were placed in the bottom of the tank at either end against the walls, and a third camera was placed 50 cm above the water surface of the tank (Figure 5). Videos used a recording rate of $29.97 \text{ frames s}^{-1}$ with an image resolution of 1920×1080 pixels.

Data synchronization

To ensure that all the data streams could be re-synchronized, we used a synchronization procedure at the end of the experiments. First, the accelerometers were gently taken off the lobsters and placed on the sand in the middle of the tank, and the two lobsters were returned to their holding tanks. Then, five sharp raps were made on the tank walls that could be used to synchronize all three types of recording device (hydrophones, accelerometers and GoPros).

Experimental design

Experiments were performed during June and August 2018 in the experimental tank described above. During each experiment, seawater temperature was measured using a HOBO Pendant G data logger (UA-004-64, Onset Computer Corporation). Seawater temperature in the experimental tank was $17.11 \pm 0.14^{\circ}\text{C}$ (mean \pm s.d.) in June and $18.44 \pm 0.12^{\circ}\text{C}$ in August.

Agonistic encounters were set up between two categories of lobsters: size-matched male lobster pairs (difference in carapace length, $\Delta\text{CL} < 5$ mm), and small and large male individuals ($\Delta\text{CL} > 5$ mm). In fact, larger lobsters are more likely to win a fight if the ΔCL is more than 5 mm between the opponents, but at smaller size differences, the outcome is random (Scrivener 1971). We formed pairs by taking one individual from each separately acclimated group to ensure that the individuals had no prior knowledge of each other (Karavanich and Atema 1998). A total of 12 agonistic encounters (6 with $\Delta\text{CL} < 5$ mm; 6 with $\Delta\text{CL} > 5$ mm) were set up.

Because communal holding causes a general reduction of aggressiveness in lobsters (Breithaupt and Atema 2000), we isolated the two selected individuals separately for 24 h in glass sided rectangular tanks ($0.60 \text{ m} \times 0.50 \text{ m} \times 0.35 \text{ m}$; 0.105 m^3) after the accelerometers were attached. This allowed the lobsters to recover from handling. For this step, the bands on their claws were also released. Lobsters were not fed during this period.

The next day, these same individuals were placed in the prepared experimental tank, one on either side of the divider (Figure 5). Experiments were performed between 16:00 h and 20:00 h. Recordings started when the individuals were placed in the tank. We recorded the first 15 min as control observations of the individuals while they were in isolation in their respective compartments. Next, we lifted the divider and continued recording the agonistic encounters that ensued for another 15 min. This corresponds to the expected minimum time for determining the outcome, according to Scrivener (1971). After the experiment, the accelerometers were removed from both animals, and the lobsters were returned to their holding tanks. Then, the

data synchronization procedure (described in ‘Data synchronization’, above) was followed. Afterwards, the experimental tank was drained completely, thoroughly rinsed and refilled with fresh seawater, and the sand was replaced. Each individual was used only once during the study.

Data analysis

Sound data

Sound files (.wav) from the two hydrophones (30 min recordings each) were archived at the end of each experiment. They were first carefully visualized over the entire frequency band (between 0 and 78 kHz) by using the spectrogram mode in Audacity® (v2.1.1; www.audacityteam.org) to check for potential biological broadband sounds emitted by lobsters during experiments. Next, sound data were subsampled between 0 and 500 Hz and spectrograms were visualized a second time using custom-written MATLAB scripts (v9.1; The MathWorks, Natick, MA, USA). The characterization of buzzing sounds has been detailed in our earlier study (Jézéquel et al. 2018). As the aim of the experiments in the present study was to understand when these sounds were produced within the behavioural sequences, here we only report the basic descriptive statistics of the buzzing events recorded during the agonistic encounters.

Accelerometry data

Data from the accelerometers were downloaded using Open Movement GUI software (v1.0.0.37). Accelerometers record movements simultaneously on three axes as the relative change detected in gravitational acceleration, g ($1 g = 9.81 \text{ m s}^{-2}$), and carapace vibrations are known to have the same frequency as their associated buzzing sounds ($\sim 100 \text{ Hz}$; Henninger and Watson 2005). After examination of the data on all three axes, we observed the strongest signals of the carapace vibrations were recorded on the x-axis, as expected. We thus used only the x-axis data to facilitate their detection among all the other high-amplitude signals related to

the lobster movements (e.g. tail flips) by treating the data using a custom-written MATLAB script. We report here the number and timing of carapace vibration events recorded during each agonistic encounter for each individual. We also measured their duration (in ms) and peak frequency (in Hz).

Video annotation of movements during encounters

Video analysis consisted of annotating the visible movements performed by each individual during the encounters. Based on the extensive *H. americanus* literature (see Table S1) and preliminary tests, we built a description of movements (also termed ethogram) by annotating 30 associated movements for five different body parts (antennae, claws, legs, carapace, tail; Table 4). We focused on movements or actions initially instead of ‘behaviours’ because it allowed us to avoid subjective choices related to the sometimes ambiguous behaviours defined in the literature. Movement directions like ‘walking away’ and ‘walking backward’ were identified according to the direction of the body axis relative to the other individual. For example, ‘walking away’ for a lobster was defined as the direction of its rostrum that pointed away from its opponent, but does not necessarily mean it was escaping from its opponent. These 30 movements were annotated for each individual and for all 12 agonistic encounters using the tools in BORIS (v6.3.9; Friard and Gamba 2016).

Video data from each agonistic encounter comprised video recordings (30 min each) from each of the three cameras used in the experiments. We chose to annotate primarily videos from the plan view camera because this covered the entire experimental area and most of the movements were visible. We completed these observations by analyzing the recordings from the two cameras placed in the bottom of the tank. This permitted us to visualize more precisely certain vertical movements made by the lobsters (e.g. high on legs, meral spread). All these annotations were then integrated with the annotation from the plan view camera for subsequent data treatment. Time energetic budgets were made for each movement and each individual

(submissive and dominant) as percentages of the total length of the agonistic encounters (15 min).

Table 4 : Ethogram of adult male European lobsters (*Homarus gammarus*) during agonistic encounters. The terms are described with respect to the direction of the movement performed by body appendages, and are not related to behaviours or ranks of aggression used in the *H. americanus* literature (see Table S1: <https://jeb.biologists.org/content/223/4/jeb211276.supplemental>). The description was used to annotate movements from lobsters in videos of agonistic encounters.

Appendage	Movement	Description
Antennae	Antennae backward	Both antennae are positioned backward along the main body axis
	Antenna pointing	One, or both, antennae directed forward, toward opponent
	Antenna sensing	One, or both, antennae directed at opponent with lateral movements
	Antenna touching	One, or both, antennae continuously touching opponent
	Antennae up	Antennae pointing directly up, perpendicular to main body axis
	Antenna whipping	Lashing of opponent's body with antenna(e) in a sweeping motion
Claws	Claw boxing	One claw is pulled backward and then makes a quick 'hook' type strike directed toward the opponent's claw(s) or body
	Claws extended	Claws rest on substrate (in front of animal) and are stretched forward so that merus-carpus-propodus of both claws are aligned with body axis
	Claw forward	One claw is stretched forward (as a weapon) while the other is held close to the body (as a shield)
	Claw grasping	Clamping of claws onto opponent's claw(s) or body
	Claw locking	Crusher claws interlocked; resembles 'handshaking'
	Claws lunging	Thrusting claws forward
	Claw open	The dactyl of either, or both, claws fully open; generally crusher claw
	Claw pushing	Continuous pushing with claws on opponent's body
	Claw ripping	Rapid grasp and pull motion, with either claw, of opponent's claw(s) or body
	Claw scissoring	Both claws pulled backward and rapidly crossed in front of opponent's claw(s) or body in a scissor-like motion
	Claw snapping	Rapid opening and closing of seizer claw in direction of opponent
	Claws touching	Continuous touching of opponent with claws
	Meral spread	Both claws held wide apart above substrate facing opponent
Legs	High on legs	All legs are fully extended raising body high above substrate
	Sand removing	Legs are used to remove sand, causing back and forth rocking movements of the body
Carapace	Facing	Body not moving and rostrum directed towards opponent
	Resting	Body not moving and rostrum not directed towards opponent
	Turning away	Body turns so rostrum points away from opponent
	Turning toward	Body turns so rostrum points toward opponent
	Walking away	Walking with rostrum pointing away from opponent
	Walking backward	Walking backward with rostrum directed toward opponent
	Walking parallel	Walking with rostrum parallel to opponent's body axis
Tail	Walking toward	Walking with rostrum pointing toward opponent
	Tail flipping	Rapid abdominal contractions which propel the lobster backward

Statistical analyses

Statistical analyses were performed using R v3.5.1 (<http://www.R-project.org/>). The mean percentage total time for each movement was tested for significant differences between dominant and submissive individuals in all 12 encounters. As these data were not distributed normally (Shapiro–Wilk test, $P < 0.05$), the non-parametric Mann–Whitney test (U-test) was used to determine whether their probability distributions were equal. The significance level for null hypothesis rejection was $\alpha < 0.05$. These results permitted us to associate sequences of movements typically shown by dominant and submissive individuals to particular behaviours based on the conventions used in the *H. americanus* literature (see Table S1).

Analysis of carapace vibration sequences

As our experiments were necessarily brief to avoid injury (15 min long; Scrivener 1971) and each experiment was also unique, the carapace vibration sequences did not meet the criteria for classical statistical tests (Guarini et al. 2019). Because the development of a behavioural model was beyond the scope of the present work, we only considered whether the sequences of carapace vibrations recorded by the accelerometers on each individual during the agonistic encounters could not have been produced by a random process. Instead of classical tests, we used a definition of randomness for algorithmic complexity that was recently formalized for short series of fewer than 100 characters that are common in behavioural studies (Soler-Toscano et al. 2014, Zenil et al. 2015preprint, Gauvrit et al. 2016).

Algorithmic complexity offers an alternative means to evaluate the existence of ordered patterns in short sequences by assessing the computing effort needed to stimulate them (Zenil et al. 2018). The approach compares a given string with results from randomly selected Turing machines calculating the likelihood that the string could be reproduced by these algorithms. In this definition, a low complexity string has a higher probability of being generated by a

randomly selected Turing machine, and therefore is less likely to have been produced by a random process (see development in Gauvrit et al. 2016). This has the double advantage of producing invariant estimates of complexity for a given observed sequence and that each experiment is treated as unique. In other words, each sequence is only compared with its own realization relative to the Turing machine algorithm. This method does not use thresholds to infer randomness (Zenil 2015). Instead, it estimates the algorithmic complexity (AC) and an indicator of the computing time required to compress the sequence structure, called the logical depth (LD; Zenil et al. 2018). A longer LD means a non-trivial structure has been found in the sequence.

To apply this method, carapace vibration sequences produced by individuals during the same agonistic encounter were transformed to time-ordered, discrete binary series. Carapace vibrations were assigned to 1, if produced by the dominant individual, or 0 if produced by the submissive individual (e.g. 1000000001010010); the rhythm of the carapace vibrations (i.e. the time between vibrations) and their duration were not represented. This also means we considered that two individuals produced carapace vibrations sequentially (i.e. as ‘call-and-response’) and not simultaneously. Because of the short length of our strings (from 14 to 98 characters), we used the block decomposition method made available through an online tool to access the necessary range of Turing machine states (Soler-Toscano et al. 2014, Zenil et al. 2018, <http://complexitycalculator.com/index.html>, v3.0). The most conservative settings were used: the largest available maximum block size (12), with no overlap and a two-character alphabet. As the AC and the LD both depend on string length, we report normalized values (as bits per character and steps per character, respectively). Hence, a standardized AC value of 1 or higher would be considered as not differentiable from random. Using a two-character alphabet, when the standardized LD is about 2 or higher, then the process that generated the sequences cannot be distinguished from a random one (Zenil et al. 2018).

Ethical note

Experiments with *H. gammarus* are not subject to restriction for animal scientific research according to the French legislation and the European Community Council Directive of September 2010 (2010/63/UE). We nonetheless followed the ARRIVE guidelines (Kilkenny et al. 2010) for all the experiments. The animals' health state was checked daily by the authors and the aquariology team of Océanopolis. During experiments, we planned to stop the agonistic encounters between two lobsters before any injury occurred to the animals; this never happened and no lobsters were injured or died during the study. At the end of the experiments, all animals were released back into the area from where they were collected.

Results

Description of the movements performed by male *H. gammarus* during agonistic encounters

When isolated on either side of the divided tank, lobsters wandered freely around the space and did not show any particular movements related to the other individual. When the divider was lifted, the individuals quickly engaged physically in an agonistic encounter (e.g. Figure 6). Initially, they made a short (< 1 min) series of threat displays, typically consisting of: antenna pointing or antenna whipping, claw open, meral spread and high on legs movements. Next, they advanced rapidly with different types of physical claw contact to drive away their opponent. This stage was mainly dominated by claw pushing movements. In 6 of the 12 agonistic encounters (4 with $\Delta CL > 5$ mm, 2 with $\Delta CL < 5$ mm), the outcome was decided at this stage. In the six other trials, the lobster pairs increased the intensity of the fight by using a variety of claw movements to attack their opponents. These movements, such as claw boxing, claw ripping or claw snapping, were very short in duration and occurred in association with aggressive upward directed tail flipping. Generally, after these actions, one individual withdrew and assumed the submissive role for the remaining time (Figure 6).

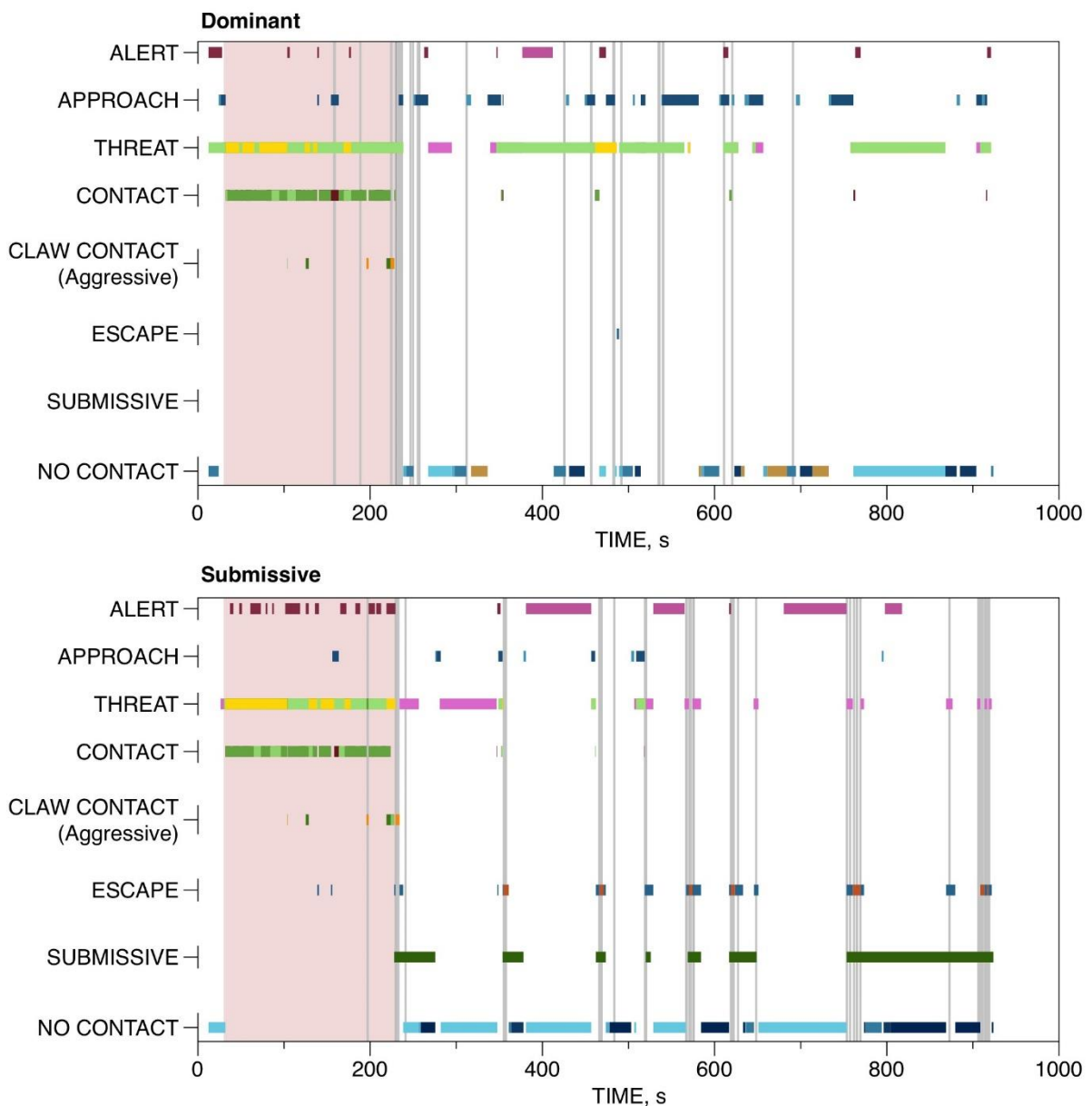


Figure 6: Example chronology of movements (horizontal colour bars) performed by two lobsters during an agonistic encounter. Top, the dominant individual; bottom, the submissive individual (difference in carapace length, $\Delta CL < 5$ mm). The different bar colours refer to different movements performed by lobsters that are shown in Table 4 and Table 5. All movements were regrouped using the behavioural terms obtained in Table 2. The first encounter is shaded red. A total of 98 carapace vibrations (vertical grey bars) were produced by both lobsters during this experiment (dominant: 35; submissive: 63), whereas only 7 associated buzzing sounds (7.1%) were recorded by the two hydrophones. Note that most carapace vibrations were produced just after the first encounter and during episodes of approach/escape between dominant and submissive individuals.

After this first encounter, which determined the hierarchical status between the two lobsters, each dominant and submissive individual displayed typical groups of movements (Figure 6, Table 5). Dominant individuals continued to perform physical displays (i.e. meral spread, high on legs and claw open), and often approached the submissive individuals (i.e. walking toward) to re-engage in physical contact (mainly antenna whipping and claw pushing). In contrast, submissive individuals always responded by escaping through physically demanding movements such as walking backward and tail flipping (Figure 6, Table 5). In particular, submissive individuals used a characteristic submissive posture with the claws extended in front of the animal for much of the period following the first encounter. Finally, when individuals were not making claw contact, the dominant animals were moving actively around the tank such as walking or sand removing, while in contrast, the submissive ones were relatively immobile (i.e. resting) near the tank walls with their claws extended (mean: 44.9% of time; Figure 6, Table 5).

Table 5 : Overview of the movement assignments to behaviours and the time budgets for the 12 agonistic encounters. Mean (\pm s.d.) percentage of time in each movement is shown for the dominant and submissive individuals. Total time was 15 min for each encounter. Bold highlights significantly different means between dominant and submissive animals (U-test, $N_1 = N_2 = 12$, $P < 0.05$).

Behaviour	Movement annotated	Dominant (% time)	Submissive (% time)
Alert	Antenna sensing	4.2 (6.2)	17.3 (14.2)
	Antennae up	10.0 (7.4)	5.8 (9.4)
Approach	Turning toward	8.9 (3.4)	2.3 (1)
	Walking toward	25.7 (7.3)	3.8 (2.5)
Threat	Antennae backward	9.6 (9.7)	2.0 (3.7)
	Antenna pointing	20.2 (8)	21.9 (16.9)
	Claw forward	3.5 (3.2)	1.7 (3.5)
	Claws lunging	2.3 (2)	1.5 (2.2)
	Claw open	31.6 (21.2)	6.9 (6.6)
	High on legs	34.9 (13.5)	7.1 (7.7)
	Meral spread	35.3 (11.3)	2.8 (9)
Physical contact	Antenna touching	0.8 (1.1)	5.7 (7.8)
	Antenna whipping	11.7 (7.8)	2.1 (3.2)
	Claw pushing	7.8 (6.5)	3.9 (5)
	Claw touching	2.6 (2.2)	0.7 (1.2)
Aggressive claw contact	Claw boxing	0.1 (0.2)	0.1 (0.1)
	Claw grasping	0.3 (0.9)	0.2 (0.4)
	Claw locking	0.1 (0.2)	0.1 (0.2)
	Claw ripping	0.1 (0.1)	0.03 (0.06)
	Claw scissoring	0.1 (0.1)	0.1 (0.2)
	Claw snapping	0.02 (0.06)	0.05 (0.1)
Escape	Tail flipping	0.2 (0.3)	2.4 (2)
	Walking backward	1.0 (0.8)	18.9 (5.6)
Submissive	Claws extended	0.0 (0)	44.9 (15.7)
No contact	Facing	16.0 (11.8)	6.1 (10.6)
	Resting	12.3 (6.7)	44.8 (11.7)
	Sand removing	5.2 (7.2)	0.0 (0)
	Turning away	4.1 (1.9)	3.8 (1.8)
	Walking away	5.8 (3.8)	9.7 (5)
	Walking parallel	7.5 (7.5)	2.8 (3)

Buzzing sounds and carapace vibrations produced during agonistic encounters

During the agonistic encounters, we did not record any particular sounds other than the buzzing sounds with the hydrophones. We identified a total of 65 buzzing sounds from 9 of the 24 lobsters tested. In marked contrast, the accelerometer data showed that 23 out of the 24 lobsters tested vibrated their carapace during the agonistic encounters. The only lobster that did not vibrate its carapace was a dominant individual. From these 23 lobsters, a total of 422 carapace vibrations were recorded, meaning that only 15% of the associated buzzing sounds were recorded by the two hydrophones in the tank. Figure 7 shows an example where two lobsters produced three carapace vibrations during a short period (6 s), and the associated buzzing sounds were only recorded by the closest hydrophone (< 20 cm from the animals). However, in most other cases when lobsters vibrated their carapaces, the associated buzzing sounds were not recorded by the two hydrophones at the same time.

We therefore used the number of carapace vibrations as a proxy for the number of buzzing sounds produced by lobsters. No carapace vibrations were detected when lobsters were first separated from each other by the divider. Even though some first encounters were long (up to 3.38 min) with highly aggressive movements between lobsters (e.g. claw ripping), very few carapace vibrations (4.7 %) were produced at this time (Figure 6). In contrast, carapace vibrations were mostly (95.3 %) produced after the first encounter (i.e. after hierarchical status was determined), during the stage of repeated approaches by the dominant individuals making threat displays towards the submissive individuals (Figure 6).

Figure 8 describes the distribution of all carapace vibrations detected by the accelerometers according to dominant or submissive outcomes. Overall, dominant individuals emitted about half as many carapace vibrations as submissive ones (141 and 281, respectively). Carapace vibrations had durations that varied from about 50 ms to nearly 600 ms, and their

peak frequencies varied between 100 and 200 Hz. No carapace vibrations were recorded that began at exactly the same time.

These data were also plotted as time series for all 12 encounters (Figure 9). There are few clear patterns in the series. The total number of carapace vibrations in an experiment between individuals of nearly the same CL (Figure 9, left) tended to be higher than that in experiments where the ΔCL was > 5 mm (Figure 9, right). Submissive individuals, which were also the only individuals to assume the ‘extended claw’ pose (Table 5), produced carapace vibrations in all encounters and mostly, but not always, while in this pose (Figure 9). For most agonistic encounters, submissive individuals produced more carapace vibrations than did dominant ones; but in three experiments (Figure 9 C, H, L), the opposite pattern was obtained and the dominant animal vibrated more frequently. In one experiment, the dominant individual was silent (Figure 9 E).

As described above, the carapace vibration series were then expressed as binary, ordered sequences and analyzed for their AC and LD. The string standardized values of the AC and LD are given in Figure 9, in bits per character and steps per character, respectively. The values of both measures ($1 < AC < 3$ and $2 < LD < 4$) indicate that the carapace vibration sequences were probably the product of a random process, and by themselves cannot be assimilated to call-and-response type signaling.

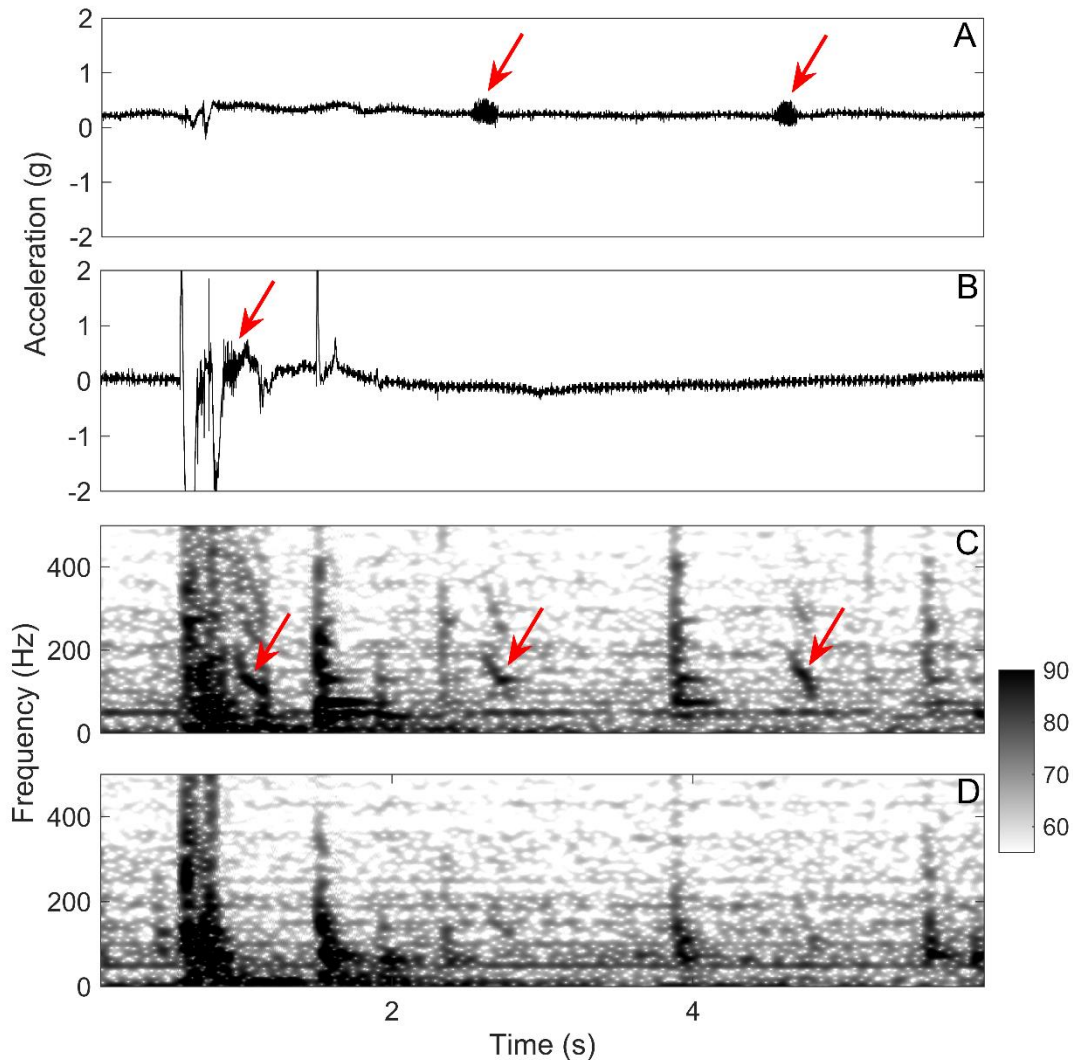


Figure 7: Synchronized data of two accelerometers (top) and the two hydrophones (bottom) during an agonistic encounter. The red arrows highlight the carapace vibrations and the associated buzzing sounds. The dominant lobster (CL = 13 cm) used claw contact and produced two carapace vibrations (A) toward the submissive lobster (CL = 11.3 cm), which escaped by tail flipping (large negative peaks) and produced one carapace vibration (B). The three associated buzzing sounds were recorded by the hydrophone located at < 20 cm from the animals (C) while the other more-distant hydrophone (D) did not record them. The vertical colour bar scale of the spectrograms is in dB re. $1 \mu\text{Pa}^2 \text{Hz}^{-1}$.

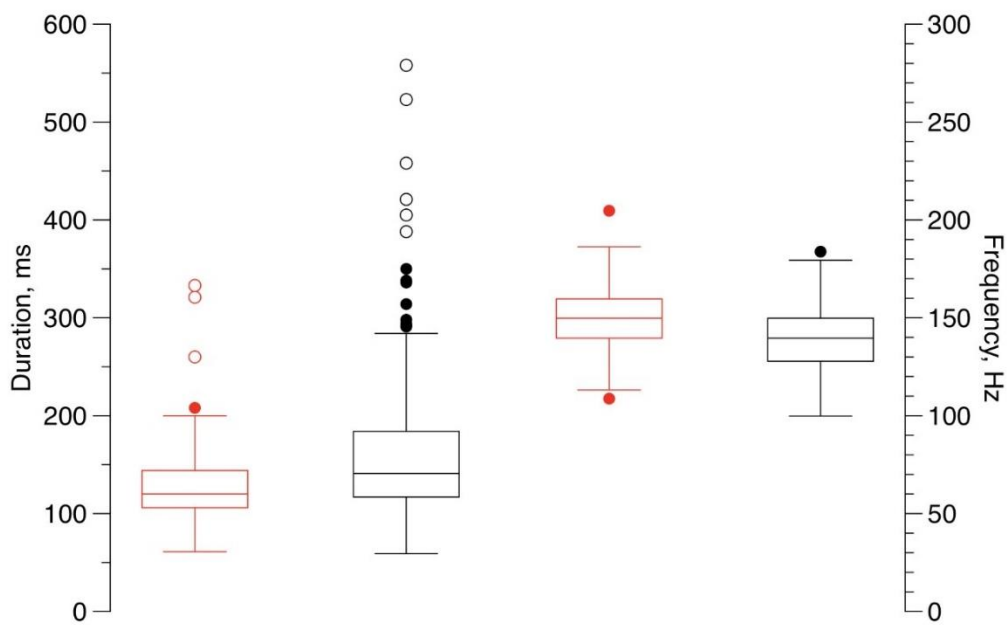


Figure 8: Box plots of carapace vibration features produced by dominant (red) and submissive (black) animals during agonistic encounters. Median values for each group are indicated by the horizontal lines. Left: duration; right: frequency.

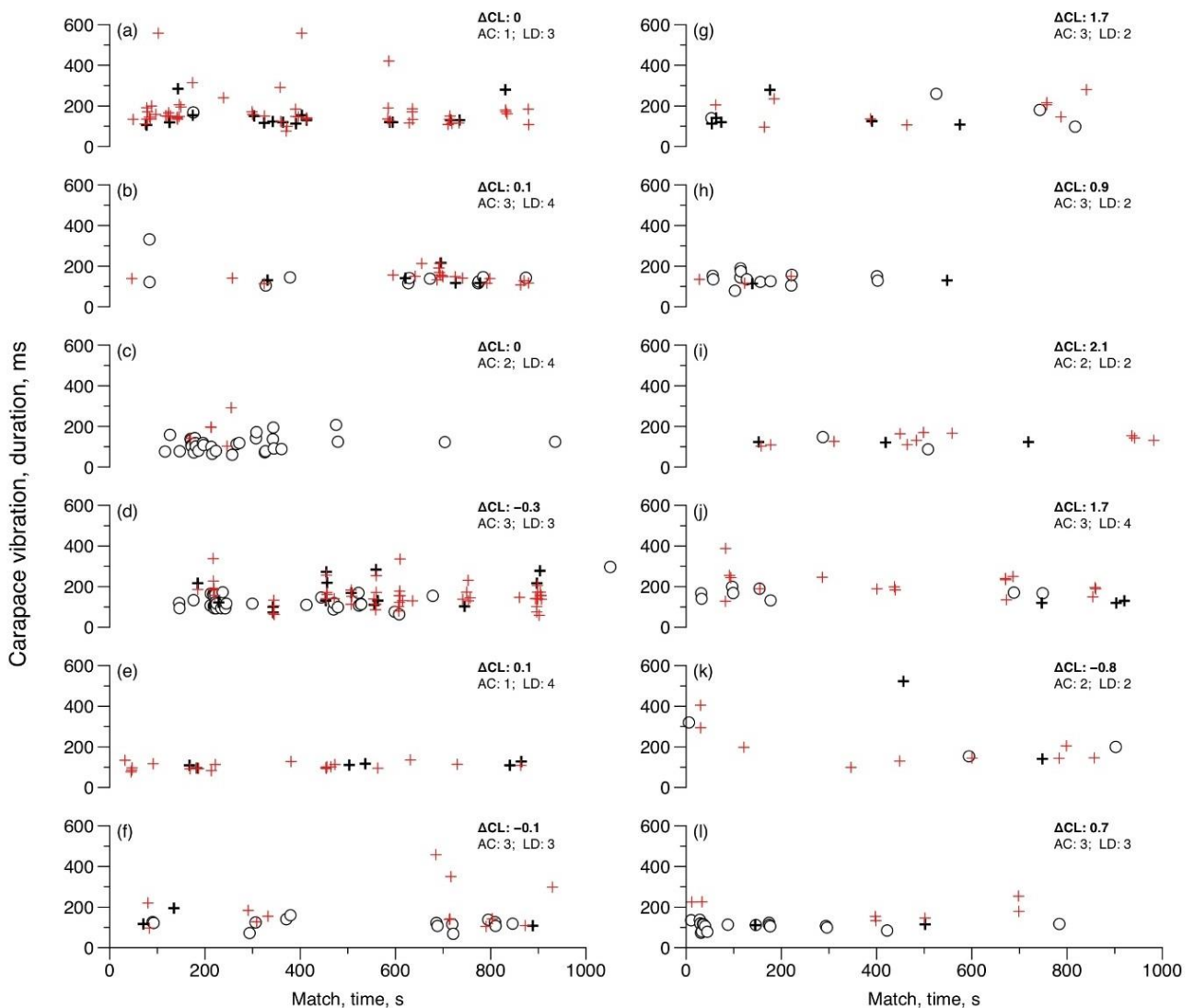


Figure 9: Emitted carapace vibrations and their duration for all 12 agonistic encounters. Dominant animals (open circles) mostly vibrated less than submissive ones (crosses). Red crosses indicate when the submissive individuals vibrated while in extended claw pose. On each plot, the relative difference in CL (ΔCL) is indicated for each pair (estimated as the difference in length of the dominant and submissive individual); the left column of plots is for the $\Delta CL < 5$ mm size class and the right column is for the $\Delta CL > 5$ mm size class. The ratios of the algorithmic complexity (AC) and logical depth (LD) standardized by the string length are also given. In our analysis, all the values indicate that the pattern of vibrations emitted by the two individuals during the encounters cannot be differentiated from a random process

Discussion

This study is the first report of male *H. gammarus* producing buzzing sounds during agonistic encounters. These sounds were produced by both dominant and submissive individuals during the experiments and were mainly emitted after the end of the first encounter (when claw contact stopped) up until the experiment ended.

Our agonistic encounters resembled descriptions of agonistic encounters published in earlier studies of male *H. americanus* (Scrivener, 1971, Atema and Voigt, 1995, Huber and Kravitz, 1995) and male *H. gammarus* (Skog et al., 2009). The initial stage consisted of a threat display between individuals that then quickly engaged in physical claw contacts, which could increase in aggressiveness (e.g. claw boxing) until the withdrawal of one individual (Figure 6). This losing individual then exhibited submissive behaviours highlighted by a claws extended pose and was less active, while the winner remained active and continued to make approaches and threat displays. At the same time, both individuals produced buzzing sounds.

However, during these experiments, very few buzzing sounds were recorded by the two hydrophones even if they were placed close to the lobsters (< 75 cm away). This is consistent with remarks made in previous studies on *H. americanus* (Scrivener, 1971, Atema and Cobb, 1980, Atema and Voigt, 1995, Ward et al., 2011). For example, Atema and Cobb (1980) stated that ‘the biological significance of such vibrations is unknown; during high intensity fights in aquaria, these sounds were rarely recorded’. Ward et al. (2011) showed, with accelerometry and sound recordings (as in this study), that the presence of another lobster in a tank significantly increased the number of buzzing sounds produced, but that these events were also rare (mean of 3 sounds per lobster in a 30 min experimental period). Nonetheless, these authors did not perform experiments concerned with agonistic behaviours between male individuals, and in addition, the accelerometers used in Ward et al. (2011) required that the lobsters were immobilized.

In the present study, we used small accelerometers which could be attached directly on the carapace where sound production occurs (Henninger and Watson 2005). This unobtrusive sensor permitted the lobsters to exhibit their full range of agonistic movements. In contrast to the earlier studies, we found that the number of carapace vibrations recorded with the accelerometers was very high during agonistic encounters. Indeed, we recorded a total of 422 carapace vibrations produced by 23 out of the 24 lobsters tested, with some individuals producing up to 70 carapace vibrations per experimental period (15 min total). In contrast, only 15% of these carapace vibrations were picked up by the two hydrophones (e.g. Figure 7). This difference in detection between hydrophones and accelerometers is explained by the high attenuation of low-frequency sounds in tanks. Although low frequencies are less attenuated than high frequencies in open water, the situation is reversed in tanks when the wavelength of the sound is larger than the tank size (e.g. a 100 Hz sound has a ~ 15 m wavelength). This phenomenon is well known in the acoustic community (Gray et al. 2016, Rogers et al. 2016), but sometimes misunderstood in the bioacoustic community. Nonetheless, it was recently highlighted through numerical simulations and empirical measurements. Indeed, Duncan et al. (2016) illustrated that the attenuation in a tank at 100 Hz is 10 dB higher than in open water (note that the exact number depends on the specific tank size and the source/receiver configuration). Moreover, Jézéquel et al. (2019) performed an empirical illustration of this phenomenon by comparing spiny lobster sounds in a tank and *in situ*. Because the high attenuation of low frequencies has been ignored in previous bioacoustic tank studies that relied on hydrophones alone, we believe that the role and importance of buzzing sounds for lobsters during agonistic encounters have been underestimated.

The detection or determination of communication amongst individual animals is a fundamental challenge in behavioural ecology (Hebets and Anderson 2018). Communication is defined most simply as a transfer of information from one or more individuals that is observed

to change the behaviour of one or more receiving individuals. Information can be transmitted and perceived in many different ways (e.g. chemically, visually, acoustically) depending on the sensory capabilities of the organisms involved. Several studies have already shown that male *H. americanus* use chemical signals as a means of communication to both recognize individuals and maintain dominance (Atema and Engstrom 1971, Karavanich and Atema 1991, Breithaupt and Atema, 2000). These same mechanisms are also known for male *H. gammarus* (Skog et al. 2009). Studies have demonstrated that the volume of urine released is closely linked with aggressive behaviours (Breithaupt et al. 1999) and, after the first encounter, only dominant individuals continue to release urine to maintain their dominance (Breithaupt and Atema 2000). There is also evidence that lobsters rely on visual signals to recognize each other (Gherardi et al. 2010, Bruce et al. 2018). All these means of communication emphasize the importance of individual-level recognition of submissive and dominant individuals. For example, this would be an advantage for avoiding additional aggressive claw contact incidents that could lead to injuries and even loss of an appendage (Breithaupt and Atema 2000).

Dominance among male lobsters also relies on their relative size differences (Scrivener 1971). In our study, 6 out of the 12 agonistic encounters were performed with closely size-matched pairs ($\Delta\text{CL} < 5 \text{ mm}$). As the encounters studied here represent examples of possible outcomes of new arrival dominance contests and not repeat encounters, the conditions should be suitable for a more important role of other signals conditioning the outcome, particularly for the encounters with closely size-matched pairs. In accordance with this, there were some intriguing patterns in the production of carapace vibrations. Indeed, we observed that submissive individuals always produced carapace vibrations, and these mostly occurred while in the claws extended pose, as well as having a broader range of duration and a higher number of carapace vibrations produced (Figures 8 and 9). In contrast, dominant individuals did not always produce a carapace vibration (e.g. Figure 9 E). We also noted that no carapace vibrations

were produced by lobsters while isolated before agonistic encounters. However, examination of the carapace vibration sequences using the paradigm of AC (Gauvrit et al. 2016, Zenil et al. 2015preprint, 2018) indicated that these sequences cannot be differentiated from a random process. As stated earlier, this could be due to their non-detection by lobsters because of the high attenuation of these low-frequency sounds in the tanks (Gray et al. 2016, Rogers et al. 2016).

When looking at sequences of carapace vibrations between the two groups of encounters with different relative CLs, the more closely size-matched pairs ($\Delta CL < 5$ mm) appeared to make a greater investment in countering the strategies of their opponents. Indeed, these encounters had more carapace vibrations, which implied more effort expended to counter the opponent's reactions (Figure 9). These preliminary results are consistent with the hypothesis that carapace vibration sequences in pairs of nearly sized-match individuals contribute to the communication of dominance, but that when size differences are larger, other signals (i.e. visual) are sufficient to establish dominance (Scrivener 1971, Atema and Voigt 1995, Skog et al. 2009). Interestingly, such multimodal communication is well known in terrestrial arthropods (e.g. Elias et al. 2006). However, we caution that as stated above, the vibration sequences cannot be distinguished from a random process and that there is a potential bias due to sound attenuation in tanks, as well as a small number of observations.

Our results emphasize not only the numerous technical challenges in these experiments but also the absence of knowledge about how lobsters may perceive sounds. For instance, in our study, the lack of a call-and-response pattern with carapace vibrations between lobsters was surprising. Indeed, individuals only produced vibrations when in the presence of a potential opponent, strongly suggesting their emission is context dependent. If combinatoriality (that is, the property of constructing meaning from apparently meaningless elements) is present, then the acoustic production would be considered communication if it can be shown to provoke a

predictable response (Engesser and Townsend 2019). This highlights the need to better understand how animals perceive sounds to be able to design appropriate experiments.

Lobsters cannot directly detect pressure from buzzing sounds, but they may still be able to detect the corresponding particle motion (Breithaupt and Tautz 1990, Breithaupt 2001, Popper et al. 2001). Indeed, a large diversity of sensory receptors dedicated to this function is known in both *H. americanus* and *H. gammarus*, including statocysts and sensory hairs (Cohen 1955, Laverack 1962). By considering this, Breithaupt (2001) suggested that lobsters may only be able to detect these sounds in the near-field, i.e. at distances less than a few tens of centimeters from the source. This hypothesis is consistent with the close-range communication well described in terrestrial arthropods (Raboin and Elias 2019). Here, we did not measure or model the acoustic particle motion field in the behavioural area as this was out of the scope of the study. As a result, if the lobsters were unable to detect the buzzing sounds using particle motion, we do not know whether this is due to the specificities of tank acoustics and/or because of biological reasons. Validating (or rejecting) this hypothesis would require further work, including model and/or measurement of near-field particle motion of lobster buzzing sounds (active and reactive intensity; e.g. Zeddies et al. 2012, Jones et al. 2019), and a better understanding of the lobster sound perception system (Breithaupt, 2001).

While some studies have confirmed experimentally the role of sound production in marine crustaceans to deter predators (Bouwma and Herrnkind, 2009, Ward et al., 2011), few studies have demonstrated these sounds are used for intraspecific communication. Interestingly, stomatopods produce low frequency sounds termed ‘rumbles’ that are similar to the lobster buzzing sounds (Patek and Caldwell 2006, Jézéquel et al. 2018). Mantis shrimps are territorial species living in burrows, like lobsters, and their sounds might help to send signals of their presence to conspecifics to maintain territory (Staaterman et al. 2011). Spiny lobsters have also been shown to emit antennal rasps during agonistic encounters in tanks (Mulligan and Fischer

1977), suggesting that these sounds may be used as a threat display. Snapping shrimps may also use their powerful ‘snaps’ to deter other conspecifics from their territory (Schmitz and Herberholz 1998). During agonistic encounters, male hermit crabs produce rapping sounds by rubbing their claws against their carapace, which may be a signal of stamina (Briffa et al. 2003). In marked contrast to other marine crustacean species where behavioural responses to sounds are not yet clear, semi-terrestrial crabs (Ocypodidae) have been shown not only to produce sounds (e.g. Taylor et al. 2019) but also to respond to these sounds during intraspecific interactions (Crane 1966, Horch and Salmon 1969, Horch 1975).

In our earlier study (Jézéquel et al. 2018), we found that *H. gammarus* produced buzzing sounds when stressed by handling. In the present study, agonistic encounters led to stressful events for both dominant and submissive individuals that resulted in the production of buzzing sounds. Thus, these sounds may be used in a similar context to the spiny lobster antennal rasps and the mantis shrimp rumbles to repel other organisms, whether conspecifics or heterospecifics (Mulligan and Fischer 1977, Bouwma and Herrnkind 2009, Staaterman et al. 2011). Taken together, these preliminary results suggest that male *H. gammarus* could use buzzing sounds, in addition to visual and chemical signals (Skog et al. 2009), as a means of intraspecific communication during agonistic encounters. However, we emphasize that our study should be repeated and include additional tests to evaluate whether these buzzing sounds really constitute communication. Other experiments should test behavioural reactions to emitted sounds as well as build an audiogram for the species associated with the quantification of particle motion (Goodall et al. 1990, Popper and Hawkins 2018). As shown in this study, because small tanks highly attenuate buzzing sounds, these experiments should be done under controlled conditions or directly in the field (Gray et al. 2016, Rogers et al. 2016). This would also be expected to change the behavioural observations. Indeed, it is not yet known at what frequency and intensity lobsters fight for dominance under in situ conditions where escape is possible (Karnofsky et al.

1989). Finally, additional studies should address the acoustic behaviour of female lobsters during agonistic encounters, as they have also been shown to be aggressive towards conspecifics (Skog 2009).

Conclusion

In this study, we have highlighted for the first time that male *H. gammarus* produce buzzing sounds during agonistic encounters. Notably, we showed that they only emitted sounds when placed in contact with each other, and that most of these sounds were produced after the first encounter (i.e. hierarchical status had been determined). However, we did not find clear evidence that these sounds could be used for communication between individuals. This may be due to the high attenuation of the buzzing sounds in tanks, which could prevent their perception by receivers. Other studies have suggested that these buzzing sounds could be a means of intraspecific communication in lobsters (Breithaupt and Tautz 1990, Breithaupt 2001). Further studies are now needed to validate this new hypothesis.

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Chapitre 3 : Détection sonore par le homard Américain



Photographie du protocole expérimental utilisé pour étudier les capacités auditives du homard Américain dans le laboratoire de neurophysiologie d'Aran Mooney au WHOI (Etats Unis).

Résumé

Nous avons montré dans le Chapitre 1 que le homard Européen produit des buzz similaires au homard Américain, puis au sein du Chapitre 2 nous avons présenté le rôle écologique potentiel de ces sons lors de rencontres agonistiques. Il apparaît alors nécessaire d'étudier leurs capacités sensorielles vis-à-vis de ces sons pour renforcer (ou réfuter) l'hypothèse de communication intraspécifique chez les homards Européens et Américains.

Globalement, les capacités auditives des crustacés marins sont peu ou pas connues selon les espèces considérées. Aujourd'hui, trois différents types de récepteurs sensoriels, externes et internes, ont été répertoriés dans la littérature bioacoustique chez le homard : les poils sensoriels couvrant leurs cuticules, les organes chodotonaux et les statocystes (voir description aux pages 5 et 6 de l'Introduction Générale). Tous les trois pourraient être sensibles aux sons basses fréquences (< 1 kHz ; Cohen and Dijkgraaf 1961, Bush and Laverack 1982, Budelmann 1992). L'organe le plus étudié dans la littérature bioacoustique des crustacés est le statocyste, qui semble pouvoir détecter les sons basses fréquences chez les crevettes et crabes nageurs (Lovell et al. 2005, Radford et al. 2016). Cependant, le (ou les) organe(s) capable(s) de détecter les sons chez les homards reste(nt) à découvrir.

Dans ce contexte, nous avons proposé puis développé au WHOI (MA, USA) une collaboration avec le laboratoire d'Aran Mooney, spécialiste de l'écoute des sons chez les animaux marins (mammifères, oiseaux et invertébrés). Le but de notre étude était d'étudier les capacités auditives du homard Américain. Ce travail a été réalisé via une approche neurophysiologique utilisant des microélectrodes pour enregistrer les potentiels d'action liés à une réponse du cerveau lorsque des sons sont imposés aux individus testés. Cette technique a déjà été utilisée chez les céphalopodes (Mooney et al. 2010) et même chez certains crustacés (Lovell et al. 2005, Hughes et al. 2014, Radford et al. 2016). Elle a permis de montrer que ces

invertébrés détectent principalement les basses fréquences, avec la meilleure sensibilité autour de 100 Hz.

Dans cette étude, nous avons pu enregistrer les réponses neuronales des homards Américains lorsqu'ils étaient exposés à des sons monochromatiques. Pour ce faire, nous avons placé des micro-électrodes à quelques millimètres de leur cerveau. Les réponses mesurées, représentées sous la forme de sinusoïdes, étaient similaires à celles décrites dans la littérature pour des crustacés et des poissons (e.g. Egner and Mann 2005, Radford et al. 2016). Divers tests de contrôle ont également été réalisés. Aucune réponse n'était enregistrée sur des homards morts, ni lorsqu'on plaçait les électrodes ailleurs que près du cerveau chez des homards vivants, ou encore en suspendant les électrodes dans la colonne d'eau. Enfin, lorsque la température corporelle est maintenue basse dans une cuve dont l'eau présente une température voisine de 4 °C, l'amplitude des potentiels d'action était plus faible et le temps de latence des réponses neuronales était plus important. Ces résultats sont similaires à ceux acquis précédemment sur des études effectuées sur d'autres espèces de crustacés (Young et al. 2006). Notre étude a d'abord ainsi pu démontrer que le homard Américain est effectivement capable de détecter des sons.

Puis, plusieurs expérimentations ont été mises en place pour déterminer quel organe sensoriel est responsable de la détection des sons chez le homard. En ablatant leurs antennules (et donc leurs statocystes), il était toujours possible d'enregistrer des réponses neuronales avec les mêmes caractéristiques que lorsque les antennules restent intègres. Nous avons ainsi rejeté l'hypothèse du rôle des statocystes dans la détection des sons chez le homard. De façon exploratoire, nous avons ensuite cherché à immobiliser les poils sensoriels présents sur la cuticule de deux homards en utilisant un vernis fixateur. Les individus présentaient alors des réponses fortement diminuées, ce qui valide le rôle des poils sensoriels dans la détection des sons. Cela confirme un ancien résultat présenté il y a plus de 50 ans par Laverack (1962, 1963).

Les poils immobilisés par le vernis, toujours en place, ne peuvent plus vibrer et donc ne détectent plus les sons arrivant au contact de la cuticule.

Pour finir, nous avons pu développer pour la première fois un audiogramme du homard Américain et démontrer que sa sensibilité auditive la plus fine se trouve dans la bande de fréquence 80 – 120 Hz, ce qui correspond à la fréquence de leurs buzz (Henninger and Watson 2005, Jézéquel et al. 2018). Ces résultats renforcent ainsi l'hypothèse du rôle écologique de ces buzz pour la communication intraspécifique chez le homard (Jézéquel et al. 2020a).

Article n°3:

Sound detection by the American lobster
(Homarus americanus)

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Abstract

While many crustaceans produce sounds, their hearing abilities and mechanisms are poorly understood, leaving uncertainties regarding if or how these animals use sound for acoustic communication. Here, we examined whether the American lobster (*Homarus americanus*) could hear and subsequently sought to discern their auditory mechanisms. Hearing responses were measured using auditory evoked potential (AEP) methods. Neurophysiological responses were obtained from the brain using tone pips between 80 and 250 Hz, with best sensitivity at 80 - 120 Hz. There were no significant differences between the auditory thresholds of males and females. Repeated controls (recordings from deceased lobsters, moving electrodes away from the brain, and reducing seawater temperature) indicated the evoked potentials' neuronal origin. In addition, AEP responses were similar before and after antennules (including statocysts) were ablated, demonstrating that the statocysts, a long-proposed auditory structure in crustaceans, are not the sensory organs responsible for lobster sound detection. However, brain AEPs could be eliminated (or highly reduced) after immobilizing hairfans which cover much of lobster bodies. These results suggest that these external cuticular hairs are likely responsible for sound detection, and imply that hearing is mechanistically possible in a wider array of invertebrates than previously considered. Since the lobsters' hearing range encompasses the fundamental frequency of their buzzing sounds, it is likely that they use sound for intraspecific communication, broadening our understandings of the sensory ecology of this commercially vital species. Yet lobsters' low frequency acoustic sensitivity also underscores clear concerns about the potential impacts of anthropogenic noise.

Key words

Marine invertebrate | Crustacean | Hearing | Auditory evoked potential | Acoustic communication

Introduction

Sound is an essential and widespread sensory cue for many marine organisms. Acoustic signals can travel efficiently underwater (Urlick 1983). It has been known for decades that marine mammals and fish use sounds to communicate with conspecifics (Tyack & Clark 2000; Ladich 2015). Repeated studies have also shown that crustaceans produce sounds (Schmitz 2002), but limited knowledge of their hearing sensitivity precludes understanding the potential uses of sound by crustaceans for intraspecific communication (Edmonds et al. 2016).

Since the first discovery of sound production by lobsters more than 60 years ago (Moulton 1957), the potential use of sound for intraspecific communication has been an intriguing area of study (Scrivener 1971; Atema & Cobb 1980; Atema & Voigt 1995; Breithaupt 2002). “Buzzing” sounds are produced by lobsters through the rapid contraction of internal muscles located at the base of their second antennae, which causes their carapaces to vibrate (Mendelson 1969). The sound features are similar in both American (*Homarus americanus*) and European (*H. gammarus*) lobsters, and are characterized by low frequencies (~100 Hz) with a relatively long duration (~200 ms; Fish 1966; Henninger & Watson 2005; Jézéquel et al. 2018). Ward et al. (2011) suggested that *H. americanus* may use these sounds primarily to deter predators such as fish. Recently, Jézéquel et al. (2020a) found that male *H. gammarus* produce repeated buzzing sounds during agonistic encounters, reviving the hypothesis for intraspecific sound communication in lobsters. However, the authors could not validate this hypothesis because there are no published data addressing whether male lobsters actually perceive sounds. Thus, there are clear needs to address sound sensitivity of lobsters.

The hearing abilities of marine invertebrates are poorly understood. Lobsters lack gas-filled organs, such as swim bladders, which are required for pressure detection (Popper et al. 2001). However, they have a variety of external and internal sensory receptors that may be responsive to low frequency sounds (Reviewed in Cohen & Dijkgraaf 1961; Bush & Laverack

1982; Budelmann 1992). Superficial receptor systems include cuticular hairfan and hair-peg organs that cover their external body surface, which have been found to be sensitive to low frequency water displacements (i.e. below 300 Hz; Laverack 1962, 1963). Chordotonal organs, which are present in the joints of body appendages, measure leg motions and are sensitive to low frequency vibrations (Bush & Laverack 1982). The most well-studied and potential organ for sound detection in lobsters is the internal sensory receptor called the statocyst, located in the basal segment of each antennule (Cohen 1955). It is a fluid-filled chamber containing sand grains, together forming a statolith, which lies in contact with sensory hairs (Cohen 1960). This receptor is primarily attributed to equilibrium and may also act as an accelerometer responding to vibrations propagated directly through a solid medium, but appears unresponsive to waterborne sounds in lobsters (Cohen & Dijkgraaf 1961). Recent studies on crabs (*Ovalipes catharus*) and prawns (*Palaemon serratus*) showed that their statocysts are also an auditory organ, with responses generated by low frequency acoustic particle motion (Lovell et al. 2005; Radford et al. 2016). Yet, for lobsters, it is not clear what organ (or organs), if any, are sensitive to waterborne sounds.

Auditory evoked potentials (AEPs) reflect synchronous neural activity as afferent responses are conducted from the auditory end-organ to the brain (Burkhard et al. 2007). AEP recording techniques have been used extensively to construct audiograms in odontocetes and fish (e.g. Fay & Edds-Walton 1997; Mooney et al. 2015). Audiograms represent the sound amplitudes (also termed thresholds) at certain frequencies above which the species are able to detect sounds. Recent AEP studies have also been done on invertebrates, including cephalopods (Mooney et al. 2010) and crustaceans (Lovell et al. 2005; Hughes et al. 2014; Radford et al. 2016). These invertebrates mainly detect low frequencies (below 1 kHz), with the best sensitivity around 100 Hz. Such a method could be useful to assess the frequency range of

response to waterborne sounds in lobsters as well as exploring which organs may transduce acoustic signals.

The aim of this study was to determine whether the American lobster (*H. americanus*) responds to sounds and the likely mechanism responsible for sound detection. Hearing range and sensitivity of *H. americanus* were measured using AEP techniques. We first sought to determine if neuronal responses could be recorded, including determining recording location. We then investigated the audiograms from both male and female lobsters and compared them with the invertebrate hearing literature to place hearing in a social and comparative context. Next, we performed control experiments to validate lobster sound detection and determine the apparent sensory organ responsible for sound detection. Finally, we discussed the ecological implications of our results for lobster ecology.

Materials and Methods

All experiments were conducted in February and March 2020 at the research facilities of the Environmental Systems Laboratory (ESL), Woods Hole Oceanographic Institution (Woods Hole, MA, USA).

Animal collection, characteristics and care

A total of 16 *H. americanus* individuals were used; they had carapace lengths (CL; measured from the eye socket to the posterior carapace margin) between 8.4 and 11.7 cm. Animals were bought from local fishermen several days after they were captured in traps; 8 males were bought in January 2020, and 8 females in February 2020. We used only intermolt individuals (as described in Aiken 1973) with full sets of undamaged appendages. Note that preliminary experiments were performed using 3 animals. These 3 lobsters were not used for the main experiments and their results were not compiled with that of the 16 individuals whose audiograms are described in this paper.

After collection, lobsters were immediately transferred to two large shaded, fiberglass circular tanks (radius = 1.1 m, effective height = 0.8 m; seawater volume = 0.77 m³) for holding in the ESL. Their claws were bound with rubber bands to avoid injury, and the rubber colors also allowed the identification of each individual lobster. The holding tanks were continuously supplied with ambient (14°C) sand-filtered seawater. One large airstone was placed in each tank to ensure high dissolved oxygen levels. Lobsters were fed with defrosted pieces of fish twice a week, and were kept under the natural photoperiod. Shelters were provided in abundance using concrete blocks, and a thin layer of sand was laid on the bottom to provide a foothold for the animals. Lobsters were acclimatized at least two weeks in these conditions before they were used in the experiments.

Experimental set-up

The AEP recordings were performed in a dedicated rectangular opaque plastic tank ($0.9 \times 0.48 \times 0.38$ m; 0.15 m³) placed in a quiet room in ESL. The experimental tank was placed inside a larger plywood box lined inside with acoustic dampening open-cell foam. The foam and wood served to reduce external noise and dampen surrounding vibrations. The box rested on rubber gaskets and a dense wooden table both of which served to further isolate the tank from surrounding vibrations. Prior to each experiment, the tank was filled with fresh, aerated, chilled seawater. The seawater temperature was measured before and after each experiment, and varied between 12 ± 0.8 at the start and 13.1 ± 0.7 °C at the end over a one-hour period, which is the optimal range of seawater temperature reported for the American lobster (Jury & Watson 2013). A UW-30 underwater speaker (Electro-Voice, Fairport, NY) was suspended, facing horizontally towards the lobster, 5 cm from the surface and 10 cm from the closest tank wall.

Prior to an AEP recording experiment, one lobster was taken from the holding tank and was attached with wires to a wooden board, ventral side down. This prevented the animal from moving during the sound exposure experiment. Preliminary trials revealed that lobsters ($n = 3$ animals that were not used for the main study) showed the strongest lobster AEP responses were obtained when placing the recording electrode (Rochester subdermal needle electrode, LifeSync Neuro, NY) near the brain. The recording electrode (diameter = 27 ga., length = 13 mm) was inserted in the basal joint of either the left or right antennule by slightly cutting the soft membrane with a scalpel. This electrode was manually inserted 3 mm beyond the carapace outer layer with the tip near the brain, and fixed to the rostrum using a wire to avoid any displacement. The location was confirmed using a dissection microscope. This was the standard location for all AEP audiogram recordings, except for other control experiments described below. In total, this procedure lasted less than one minute. Then, the lobster was suspended horizontally in the water column of the experimental tank with its dorsal carapace located 3 cm

below the surface and the anterior part of its carapace (i.e. location of the brain and recording electrode) facing the underwater speaker at a distance of 35 cm. Care was taken to position each lobster at the same distance from the speaker to enable comparisons between individuals, as low frequency sounds are highly attenuated in tanks (Jézéquel et al. 2019; Jones et al. 2020). Once situated, a reference electrode was inserted into the soft membrane in the telson, between 20 cm and 30 cm from the recording electrode. A ground electrode was also suspended in the water column of the tank.

All electrodes were modified by coating the entire stainless-steel portion (except the very tip 0.5 – 1 mm) with a thin layer of Por-15 (Morristown, NJ, USA), and their cables were coated with aluminum foil, which reduced extraneous electrical noise. The connection of the stainless-steel tip to the electrode cable was lightly coated in epoxy resin to prevent seawater from penetrating the connection. The tank was grounded using a wire connected to the outgoing seawater flow of ESL. Prior to AEP recordings, the lobsters were acclimatized five minutes in the experimental tank to recover from handling. After each experiment, the animals were returned to their holding tank. Each animal was used only once during the study. After each AEP experiment, the tank was drained completely, thoroughly rinsed and refilled with fresh seawater for the next experiment.

Auditory evoked potential recordings

The electrodes were connected to a battery-powered Grass CP-511 biological amplifier and filter (Astro-Med Inc., West Warwick, RI, USA) that amplified the signal with a gain of 40 dB and bandpass-filtered responses from 30 to 3000 Hz. The received signal was then converted from analog to digital via a National Instruments BNC-2110 data acquisition card (DAQ; Austin, TX, USA), and saved with a custom AEP program (using National Instruments LabView software) on a laptop computer. The AEP data were sampled at 16 kHz. A total

of 1000 sweeps were collected and averaged for each record. The same laptop, custom program and data acquisition card were used to generate acoustic stimuli.

Preliminary trials revealed that lobsters ($n = 3$ animals that were not used for the main study) did not respond to sound frequencies above 250 Hz (using the same set-up as in Section 2.2), even at the highest amplitudes the equipment could generate (~ 150 dB re 1 μ Pa at 35 cm). Therefore, AEP recordings were performed using amplitude modulated tone-pips of 80, 100, 120, 150, 170, 200, 220 and 250 Hz. The presentation order of the frequencies was random. The characteristics of the different stimuli played to the lobsters are presented in Table 1. Sound stimuli were played from the data acquisition card to a 350D attenuator (Hewlett Packard, Loveland, HP, USA) where sound pressure levels could be manually adjusted in 1–10 dB steps, and then to an amplifier (PLA2378, Brooklyn, NY, USA) which was connected to the underwater speaker. Measurements at 35 cm started at maximum sound pressure levels (SPLs), whose value was frequency dependent because of the characteristics of the underwater speaker (see Table 6): 110.2 – 134.9 dB re 1 μ Pa; associated particle acceleration levels (PALs) were -31.9 – -17.3 dB re 1 $\text{m}\cdot\text{s}^{-2}$.

The SPLs were then gradually decreased with the attenuator, and the corresponding AEP responses were visually monitored. The SPLs were first decreased in 5- or 10-dB increments depending on the amplitude of the AEP response, and in 2 dB increments when close to the thresholds until the stereotypical AEP response was no longer detectable. Then, one to three additional recordings at 2-6 dB below the visually-determined thresholds were made to ensure low responses were not missed.

Table 6: Features of the stimuli played to the lobsters by the speaker in the experimental tank.

Stimuli (Hz)	Duration (ms)	No. of cycles	Rec. window (ms)	Presentation rate (s^{-1})	Start SPL _{rms} (dB re 1 μ Pa)	Start PAL _{rms} (dB re 1 $m.s^{-2}$)
80	30	2.4	100	8	110.2	-31.9
100		3		8	116	-31.1
120		3.6		8	124.6	-26.6
150		4.5		10	133.4	-18.6
170		5.1		10	134.9	-17.3
200		6		10	131.3	-21
220		6.6		10	126	-25
250		7.5		10	121.6	-21.3

Acoustic calibrations

Sound pressure (SPL_{rms}, dB re 1 μ Pa) and particle acceleration levels (PAL_{rms}, dB re 1 $m.s^{-2}$) in the experimental tank were calibrated in the absence of animals. These values were calculated at the same distance as the recording electrode and lobster brain/statocysts were located from the underwater speaker (i.e., 35 cm). We chose to perform acoustic calibration at this location because the previous bioacoustic literature stated the statocysts are the sensory organs for sound detection in marine crustaceans (Lovell et al. 2005; Hughes et al. 2014; Radford et al. 2016).

Sound pressure levels were determined using one pre-amplified hydrophone (HTI-96-MIN, High Tech Inc., Long Beach, MS) with a sensitivity of -165.0 dB re 1 μ Pa $^{-1}$ and a flat response from 2 Hz to 50 kHz. The hydrophone was connected to an autonomous recorder (SoundTrap ST4300, Ocean Instruments NZ, Inc.) with a gain of 1 dB. SPL_{rms} were calculated as root-mean-square at each tested frequency and attenuation level between 50 and 300 Hz over a one-minute period.

Particle acceleration levels were estimated using a tri-axial accelerometer with a custom-built waterproof housing (Model W356B11, PCB Piezotronics; Sensitivity: X = 1.039 $mV m.s^{-2}$; Y = 1.036 $mV m.s^{-2}$; Z = 1.052 $mV m.s^{-2}$) wired through a signal conditioner (Model 480B21, Piezotronics) which multiplied the recorded voltage by a factor of 100. The accelerometer signal was input to three analog filters (one per axis; Model FMB300B, Krohn-

Hite corporation) which each applied a bandpass filter between 60 and 3000 Hz. Outputs of the filters were input to a data acquisition board (USB 6251, National Instruments), which was in turn connected to a laptop that ran a custom MATLAB (Mathworks, Natick, MA) script to record the audio files. Voltage values in root-mean square for each axis (X, Y and Z) were calibrated to the sensitivity of the accelerometer and used to calculate the magnitude of particle acceleration (PAL_{rms}) in dB and linear scale in the same frequency range as the SPL_{rms} . The sensitivity of the accelerometer did not allow us to accurately measure the PAL thresholds (lowest sound levels) at the lowest frequencies (i.e. 80 – 120 Hz). However, PALs could be measured supra-threshold at other frequencies and calculated by verifying the attenuator steps.

All calculations for SPL_{rms} and PAL_{rms} were performed with custom-written MATLAB scripts (v9.1; The MathWorks, Natick, MA, USA).

Additional experiments

After recording AEP responses for audiograms in all 16 lobsters, we performed additional experiments with the same individuals (different individuals were used for each experiment). The objectives were to perform controls 1) to verify if AEP recordings indeed indicated neural responses to acoustic stimuli, and 2) to better understand the lobster sensory organs.

Controls

To confirm the evoked potentials were neuronal in origin and in response to sound we performed two control experiments.

In the first control experiment, we performed AEP measurement experiments on dead animals ($n = 3$). The lobsters were killed by placing them in the freezer ($- 40\text{ }^{\circ}\text{C}$) for 24 hours. They were then defrosted and AEP measurements were made.

In the second control experiment, we recorded AEP responses of lobsters under very low seawater temperature ($n = 2$). First, we recorded AEP responses from the lobsters to a 100

Hz stimulus under normal (ambient) conditions (11.5 °C). Then the seawater was drained and refilled with cold seawater (4.2 °C) and AEP measurements were repeated. Finally, the cold seawater was drained and the tank was refilled with ambient seawater (~ 11.8 °C), and AEP responses were measured once more.

During these control experiments, the recording electrode was always placed at the standard location (i.e., adjacent to the brain) and AEP experiments were run using the protocol described in section 2.3.

Sensory organs

To understand the source of the AEP responses, we performed several different AEP experiments, while placing the recording electrode in other locations than the brain, based on the existing bioacoustics literature. We placed the recording electrode into the soft musculature of the carapace – abdomen junction, and in the articulations of claw and leg appendages, seeking to potentially record AEPs from chordotonal organs (n = 2; Bush & Laverack 1982).

We also examined potential contributions of the antennules to AEPs. We removed both antennules of several lobsters (n = 4). Removal of antennules was achieved by cutting (using a scalpel) their basal segments which contain the statocysts (Cohen et al. 1955). Then, lobsters were allowed one week to recover. During this period, the animals behaved normally and kept feeding. This post-ablation recovery period was included to give the lobster time to settle after this procedure, as its metabolic state soon after ablation could have had a detrimental effect on AEPs (Lovell et al. 2005). We then measured AEP responses as described above (see section 2.3).

Last but not least, we assessed the potential of hairfans as sound detection organs. To do so, we sprayed the entire body surface (including legs, claws and body, except the anterior part of the carapace) of two individuals using a lacquer spray (Rust-Oleum, Vernon Hills, IL,

USA). After the lacquer sealing, hairfans were completely solidified and could not be moved while touching by hand. The lobsters were allowed three days to recover from handling, and AEP responses were recorded.

Data analysis

Thresholds determination

We assessed auditory thresholds using two different methods. First, AEP waveforms (i.e. time series) were visually processed, a method commonly used in marine fish and invertebrate hearing investigations (Mooney et al. 2010; Hughes et al. 2014). We determined the attenuation levels at which responses were present and absent. The visual thresholds corresponded to the lowest attenuation levels where responses were still present in the AEP recordings.

These analyses were complemented by Fast Fourier transform power spectrum analysis (FFT; Hamming window: 321-561 points, depending on the length of the response) of the averaged waveforms using custom-written MATLAB scripts (v9.1; The MathWorks, Natick, MA, USA). As with fish and squid AEPs, the FFT spectra revealed peaks at approximately twice the stimulus frequency (Egner & Mann 2005; Mooney et al. 2010). The amplitudes of the FFT peaks also decreased as attenuation levels increased. These values were then plotted relative to the corresponding attenuation levels and a linear regression was calculated using this dataset. We collected between 4 and 10 values per tested frequency (mean = 5.9), and the points with the highest r^2 value were used to calculate the regression (Mooney et al. 2010). The point at which the linear regression crossed the y-axis corresponded to the theoretical attenuation level at which no AEP response would occur and coincided to the threshold at a given frequency (Nachtigall et al. 2007).

Statistical analysis

We first tested whether differences in means between the CLs of male ($n = 8$) and female ($n = 8$) lobsters were significant. As the CL data were not normally distributed in both groups (Shapiro-Wilk test, $p < 0.05$), a non-parametric Mann-Whitney (U) test was used ($\alpha = 0.05$).

The auditory thresholds data were distributed normally (Shapiro–Wilk test, $p > 0.05$). Thus, one-way ANOVAs ($\alpha = 0.05$) were used to compare differences in thresholds (SPL_{rms}) and frequencies (Hz) between: males and females ($n = 16$), visual and linear regression analysis methods ($n = 16$), and lobsters prior and after antennule ablation ($n = 4$). Statistical analyses were performed using R v3.5.1 (<http://www.R-project.org/>).

Results

AEP waveform features

Evoked potential responses were recorded from all 16 live lobsters tested during the main experiment. The evoked potential responses could be detected 30 and 40 ms following the stimulus onset (Figure 10). This latency accounted for the time that the sound traveled the 35 cm distance to the lobster, plus the neurophysiological response latency of the animal at ca. 12 °C. The AEP responses were gated sine waves easily discernable above the noise level when stimulus amplitudes were high. Their durations were close to the stimulus duration (~ 30 ms; Figure 10). The response amplitudes decreased as stimulus levels decreased. Response amplitudes were higher for a given stimulus level at frequencies of best sensitivity (80 - 120 Hz). Indeed, at these frequencies, the peak-to-peak amplitudes often reached levels near 2 μ V. All responses disappeared below the thresholds. Similar to fish and other marine invertebrates for which AEP responses have been measured, the frequency of the AEP responses corresponded to about twice the stimulus frequency (see Annex 2).

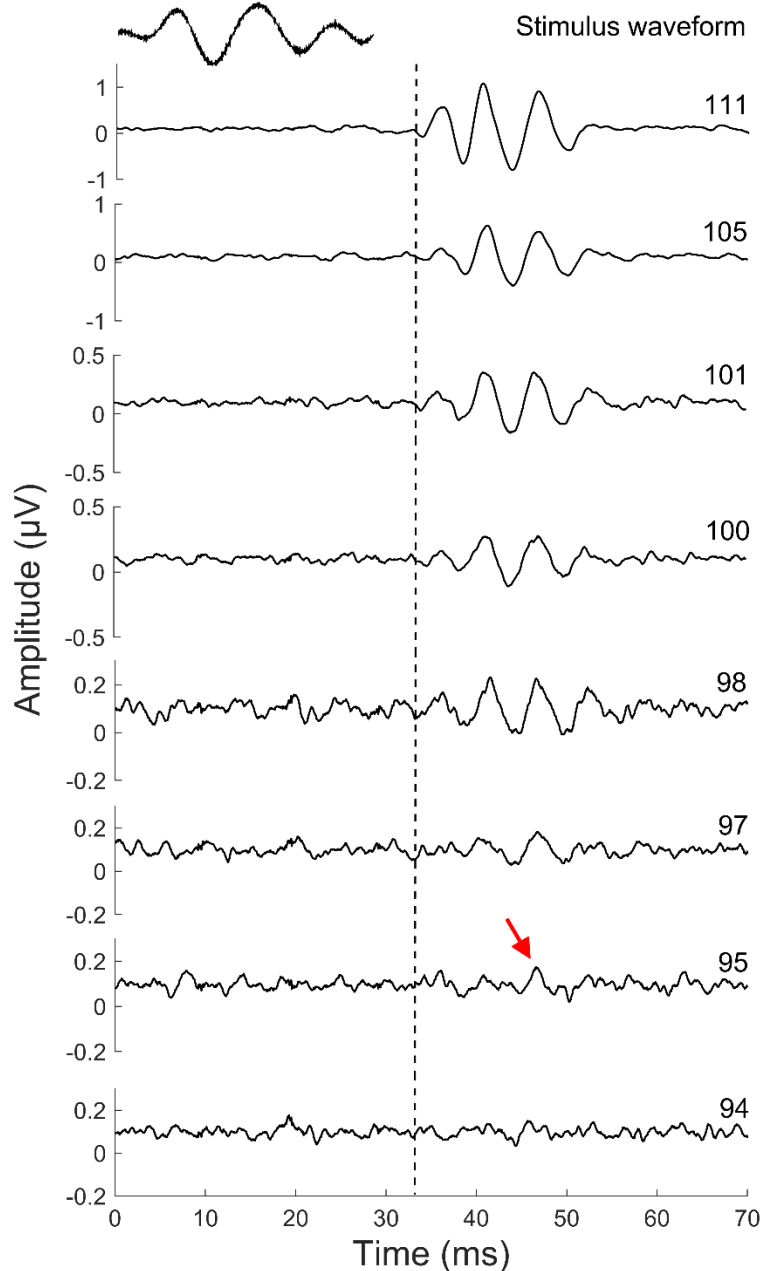


Figure 10: AEP responses from a male lobster (CL = 8.6 cm) to a 80 Hz tone-pip stimulus (waveform overlaid at the top, duration = 30 ms) with SPL_{rms} from 111 to 94 dB re 1 μPa . Each response was collected using 1000 sweep averages. Note that the vertical axes have different scales relative to the response amplitude. The peak frequencies of the observed responses were twice the frequency of the 80 Hz tone-pip stimulus (i.e. 160 Hz; see Supplementary Material). The vertical black dashed line shows the onset of the response waveforms, which represents the latency between the stimulus exposure and the responses (here the latency is around 33 ms). The red arrow highlights the lowest observed response from the lobster, and thus the visually determined threshold was 95 dB re 1 μPa .

Audiograms

The audiograms of both male ($n = 8$) and female ($n = 8$) lobsters were compared with the threshold data in units of SPL_{rms} (Figure 11). Overall, lobsters showed greater sensitivity (i.e. lower thresholds) at 80 - 120 Hz, with individuals having thresholds often below 100 dB re 1 μPa . The lowest determined threshold was 95 dB re 1 μPa at 80 Hz, and was only 3 dB above the ambient noise level of the experimental tank (Figure 10). As test frequency increased, the thresholds also elevated to 120 dB re 1 μPa at 220 Hz. Of the 16 individuals tested, only one male and two females responded to the 250 Hz stimulus. These three individuals had the lowest thresholds amongst all tested lobsters. While the mean CLs between groups of males ($n = 8$) and females ($n = 8$) differed significantly (U -test, $p < 0.05$; the male CLs were larger), we did not find any significant differences between the audiograms (One-way ANOVA, $F_{1, 12} = 0.01$, $p = 0.92$). In addition, the two threshold analysis methods (visual and FFT) provided similar audiograms in both males (One-way ANOVA, $F_{1, 12} = 0.09$, $p = 0.769$) and females (One-way ANOVA, $F_{1, 12} = 0.133$, $p = 0.722$). PAL_{rms} thresholds showed the same pattern as SPL_{rms} thresholds, and varied between -35 dB re 1 $m \cdot s^{-2}$ for lower frequencies (80 - 120 Hz) and -20 dB re 1 $m \cdot s^{-2}$ for higher frequencies (200 - 250 Hz). However, the PAL thresholds are underestimated for the low frequency band (below 150 Hz) because of the sensitivity of the accelerometer used (see Materials and Methods).

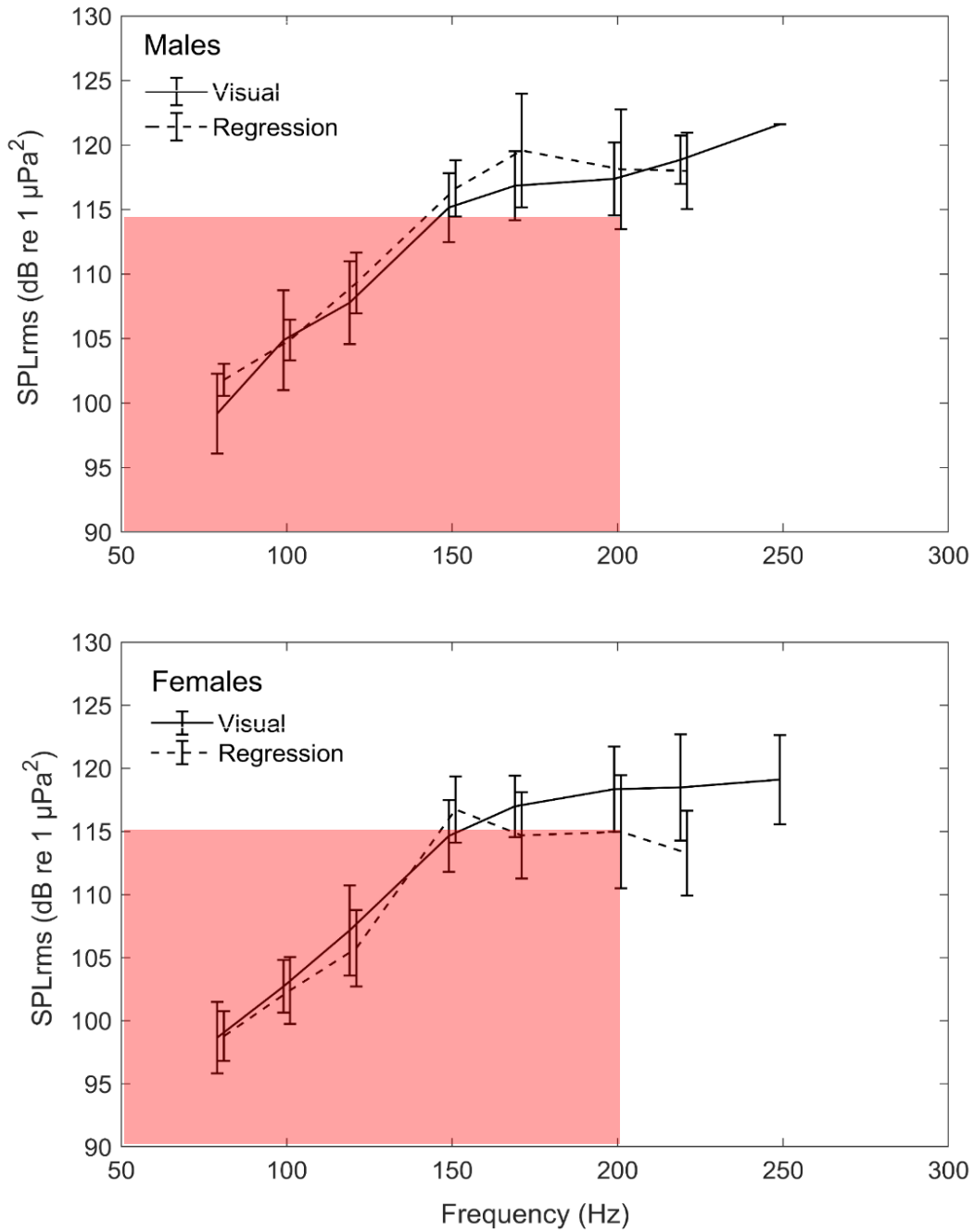


Figure 11: Auditory threshold data in units of SPL_{rms} in male ($n = 8$; top) and female ($n = 8$; bottom) American lobsters. Continuous lines: visual thresholds; dashed lines: linear regression thresholds. The red area shows the intensity and frequency range of the buzzing sounds produced by lobsters (Fish 1966, Henninger & Watson 2005, Jézéquel et al. 2018). Vertical lines are standard deviations.

Additional experiments

The AEP responses were obtained under several different control situations to confirmed neural responses of the lobsters to sound, and to assess their potential hearing organs.

Controls

No responses were obtained from dead animals ($n = 3$), nor from placing the recording electrode in locations other than adjacent to the brain of live animals (Figure 12).

The effects of temperature on AEPs were investigated in two lobsters at 100 Hz (the frequency of maximal response amplitudes). Initial recordings were first done at 11.5 °C to assess baseline AEP response levels and confirm that the response characteristics were similar to those previously established (Figure 13). Then, lobsters were placed in cold seawater (4.2 °C) and responses were measured. The recorded response waveforms were different compared to the baseline previous AEPs: the peak-to-peak amplitudes were 3 to 6-fold lower and the latencies were more than 20 ms longer. When the lobsters were returned to the baseline, acclimation seawater temperatures (11.8 °C), their response amplitudes and latencies returned to their initial levels (Figure 13).

Sensory organs

Surprisingly, the four lobsters tested after ablating their antennules (including the basal segments and statocysts) presented clear AEP responses, similar to normal lobsters (Figure 12). Indeed, we did not find any significant differences in the audiograms of these lobsters prior and after ablating their antennules (One-way ANOVA, $F_{1, 12} = 0.032$, $p = 0.862$). This indicated that the statocysts were not the sensory organs responsible for lobster sound detection. Interestingly, when hairfans were immobilized using lacquer spray ($n = 2$), the AEP responses from the brain were either extinguished or highly reduced in amplitude (Figure 12), leading to the suggestion that these hairfans play a role in hearing.

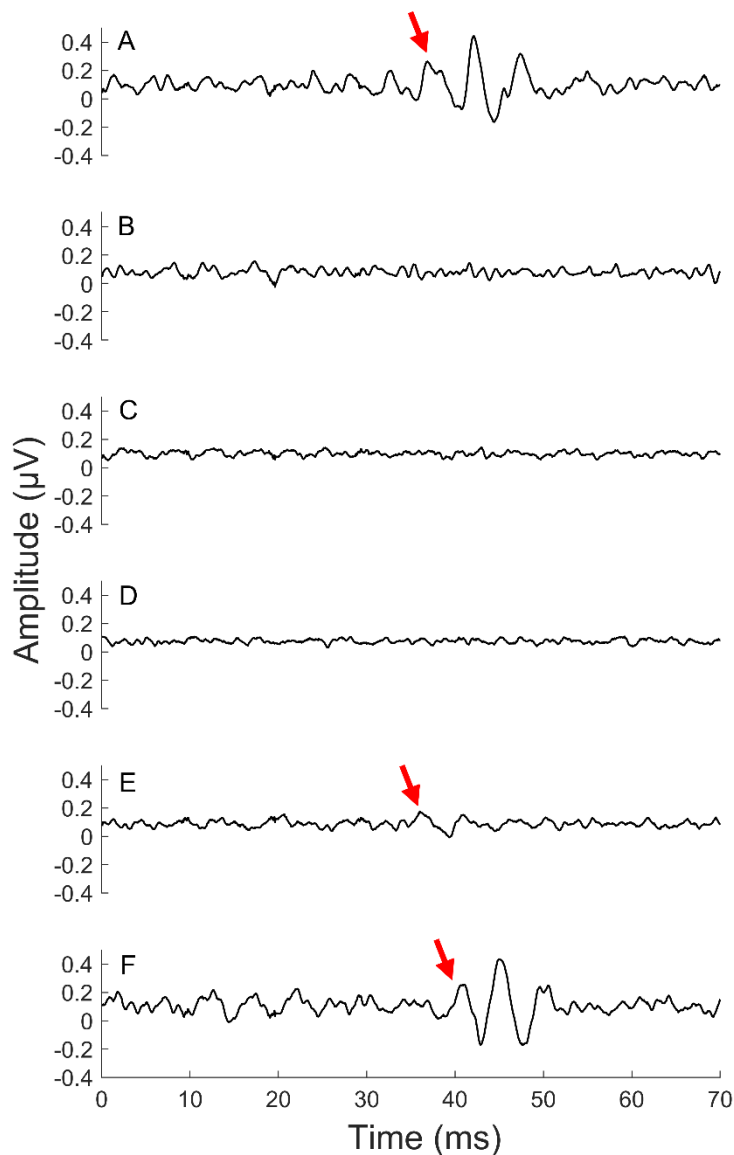


Figure 12: Lobster evoked potentials to a 100 Hz tone-pip at 111 dB under different control experiments. (A) AEP from one animal in the standard recording location (near the brain). (B) The electrodes were suspended in the water without the animal. (C) The recording electrode was moved at the carapace – tail junction into the musculature. Then, the recording electrode was placed in the standard recording position but (D) animal was dead, (E) the hairfans covering lobster body were immobilized using lacquer (but antennules were not ablated) and (F) the antennules (including statocysts) were ablated (while the hairfans were intact). The red arrows show the onset of the AEP responses.

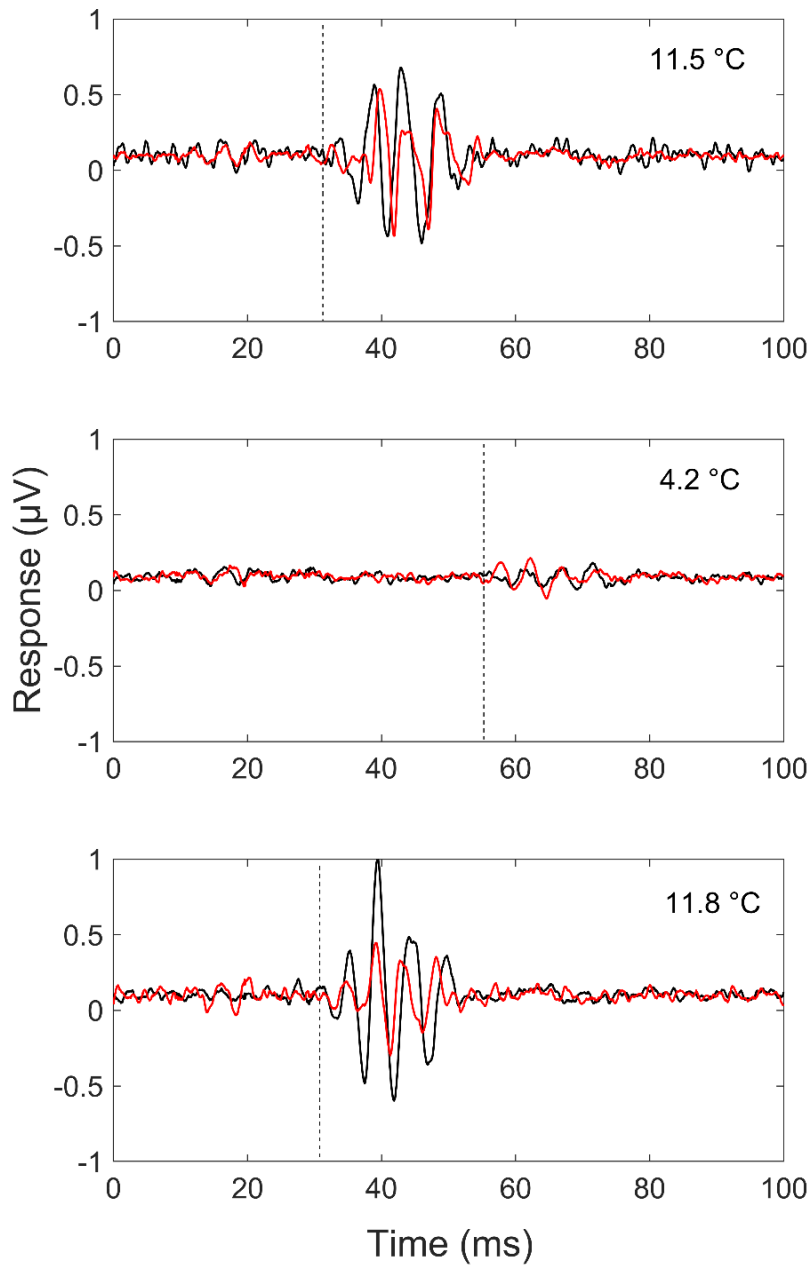


Figure 13: The AEP responses from 2 lobsters (red and black curves) exposed to consecutive different seawater temperatures: first 11.5 °C, then 4.2 °C and then 11.8 °C. Responses were to a 100 Hz tone-pip at 111 dB. The onset responses are shown with vertical black dashed lines. Note that at 4.2 °C, the AEP response latencies were more than 20 ms longer and 3 to 6-fold lower amplitude compared to acclimation conditions.

Discussion

This is the first study demonstrating sound detection in *H. americanus* using AEP methods. Lobsters detect sounds below 250 Hz with best sensitivity between 80 and 120 Hz, a range which encompasses the fundamental frequency of their buzzing sounds. These auditory data support the potential role of buzzing sounds for intraspecific communication in lobsters.

Auditory sensitivity of the American lobster and comparison with literature

Classical studies of animal audition often rely on psychophysical approaches such as behavioral responses or cardiac conditioning (Popper & Fay 1993). To our knowledge, only one attempt of *H. americanus* sound detection has been performed using cardiac assays (Offutt 1970). This study showed lobsters react via bradycardia to frequencies below 150 Hz, with best sensitivity at 75 Hz. However, heart rate responses in decapods are difficult to assess as they can be caused by many external stimuli (Larimer & Tindel 1966). Other techniques are thus needed to verify and broaden these results.

While AEP methods are well established in fish, humans and other animals, they were novel for lobsters, thus the responses required some evaluation. At the most basal level, the AEP response latencies and waveform features were clearly observable at sound levels well above thresholds (see Figure 10). Moreover, these responses did not exist when using dead animals, suggesting that the responses were not a mechanical or electrical artifact of the stimulus (Figure 12). In addition, the response frequencies were about twice the stimulus frequencies (see Annex 2), as seen in other invertebrates (squids: Mooney et al. 2010; crabs: Hughes et al. 2014) and fish (Egner & Mann 2005; Rogers et al. 2020). This has been explained to be a function of hair cells that are oriented (and maximally stimulated) in-line and in the opposite phase but parallel to the direction of the acoustic waves (Fay 1974). Thus, the current data suggest a similar mechanism in lobsters. It is reasonable to consider here that hairfans may

generate responses at twice the stimulus frequency, given their mechanosensory roles (Laverack 1962).

Overall, the latency of AEP responses in lobsters ranged between 30 and 40 ms following the stimulus onset, a result consistent with other AEP studies (Mooney et al. 2010; Rogers et al. 2020). Interestingly, we still recorded AEP responses when placing the lobsters in cold seawater (4.2 °C). However, the waveform amplitudes were highly reduced and the latencies were 20 ms longer compared to AEP recordings under warmer, ambient conditions (11.5 °C; Figure 13). These results are consistent with previous studies performed in other temperate marine crustaceans. Young et al. (2006) showed that both neuronal conduction velocity and response amplitude of axons in the leg nerves of *Carcinus maenas* and *Ligia oceanica* decreased with temperature. Taken together, these results provide further evidence that we recorded neuronal responses from a sensory organ reacting to sounds rather than a physical artifact related to animal body vibrations to water particle motion. Overall, our results clearly demonstrate that lobsters are capable of detecting low frequency sounds.

Studies presenting hearing abilities of marine crustaceans are scarce. Comparing hearing studies can be challenging given the potential effects of different recording techniques, physical set-up, and the lack of consistency in reported threshold units (Ladich & Fay 2013). Comparisons should be therefore considered an opening discussion. We compared our results with three other studies that also reported visually determined AEP hearing thresholds in three different species of marine crustaceans (see Figure 14): prawns (*Palaemon serratus*; Lovell et al. 2005), mud crabs (*Panopeus* spp.; Hughes et al. 2014) and paddle crabs (*Ovalipes catharus*; Radford et al. 2016). While we found lobsters detect sound only below 250 Hz, prawns and crabs detect sounds up to 3000 Hz (Lovel et al. 2005; Hughes et al. 2014; Radford et al. 2016), an order of magnitude difference. However, the band of best-sensitivity in terms of both SPLs and PALs (i.e. the lowest thresholds) was similar for all four reported species. Lowest

thresholds were found at low frequencies, below 150 Hz (Figure 14a). Such a result is consistent with the bioacoustics literature stating invertebrates mainly detect low frequency sounds (Budelmann 1992), and confirms the auditory responses obtained using cardiac assays in American lobsters (Offutt 1970). While lobsters have similar sensitivity in this frequency band in terms of SPL thresholds compared to prawns (Lovell et al. 2005), there is a large gap (35 dB) at 80 Hz between lobster and mud crab thresholds (Figure 14a; Hughes et al. 2014). However, the lobster hearing thresholds in units of PALs are in the same order of magnitude as for paddle and mud crabs (Figure 14b; Hughes et al. 2014; Radford et al. 2016). While AEP audiograms give a reasonable estimate of auditory responses for lobsters, these results need to be complemented with behavioural thresholds for freely moving individuals (Kojima et al. 2005; Ladich & Fay 2013; Popper et al. 2014). We also recognize the need to standardize the protocols and set-ups to make studies in crustacean hearing directly comparable (Sisneros et al. 2016).

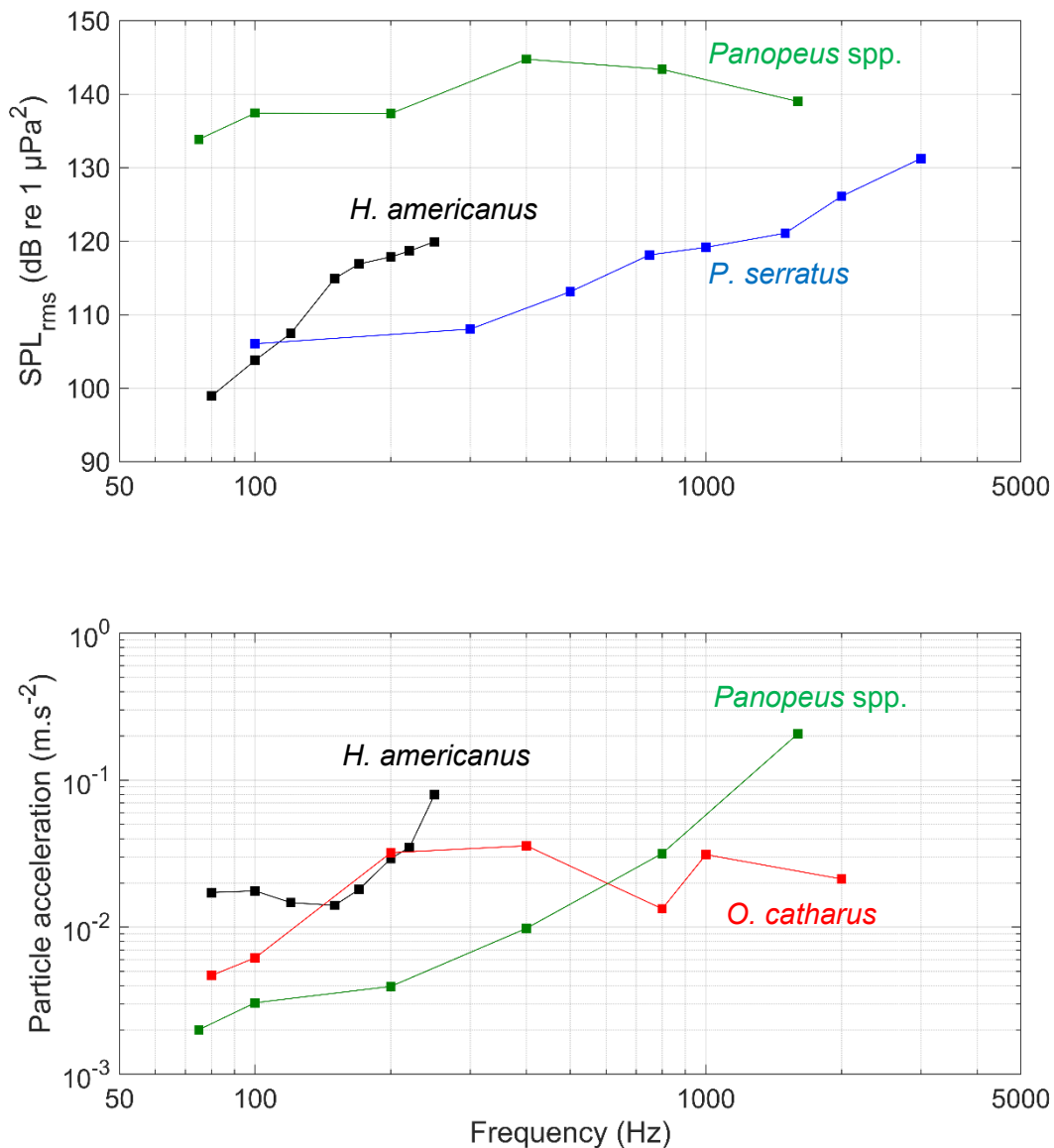


Figure 14: Audiogram of the American lobster (both males and females: $n = 16$; black lines) with auditory threshold units in SPL_{rms} (A) and PAL_{rms} (B). Superimposed on the figures are the threshold levels determined by outside water speaker as reported in Lovell et al. (2005; blue, in A) for prawns (*Palaemon serratus*), and underwater speaker as reported in Hughes et al. (2014; green, in A and B) for mud crabs (*Panopeus spp.*) and in Radford et al. (2016; red, in B) for paddle crabs (*Ovalipes catharus*). Note both frequency and particle acceleration axes are in logarithmic scale. The flattening of the lobster PAL curve (black line, in B) at lower frequencies (below 150 Hz) is due to the lack of sensitivity from the waterproof accelerometer (see Materials and Methods).

Hearing organ

Determining which sensory organ is responsible for sound detection in marine invertebrates helps illuminate how signals are perceived. The majority of recent hearing investigations in marine crustaceans has focused on statocysts as the sensory organ (Lovell et al. 2005; Hughes et al. 2014; Radford et al. 2016). Our study has demonstrated that lobsters present no significant differences in their auditory thresholds without and with their antennules (including statocysts) ablated (see Figure 12). Such a result indicates that the statocysts are not the sensory organs responsible for sound detection in lobsters. This corroborates a statement previously made by Cohen & Dijkgraaf (1961).

This apparent contradiction in the invertebrate bioacoustics literature may be explained by the experimental methods used in previous AEP recording experiments. For example, Hughes et al. (2014) performed AEPs in mud crabs by placing the recording electrode inside the carapace at the basal segments of the antennules (i.e. near the brain of the animals), as in our study. However, the authors did not perform antennule ablation, and thus cannot conclude the role of statocysts for sound perception in this species. Interestingly, Radford et al. (2016) showed that paddle crabs with crushed statocysts still respond to sounds from an underwater speaker, but not to particle motion from a shaker stimulus. The authors concluded that there may be another sensory organ in *O. catharus* that could be pressure sensitive.

Other sensory organs, termed hairfans, are found in large numbers on the body and appendages of lobsters and other crustaceans (Budelmann 1992). In this study, we found that lobsters with immobilized hairfans had highly reduced or extinguished the AEP responses (Figure 12). This suggests they play a key role in sound detection. These cuticular hairs have been already shown previously to be sensitive to water displacements below 300 Hz (Laverack 1962), which encompasses the lobster hearing range found in our study. Laverack (1962) even

indicated that these sensory organs may act as sound pressure detectors. Interestingly, these structures are not present on lobster antennae and antennules (Laverack 1963), which corroborates our results showing that AEP responses were still recorded in lobsters when their antennules were ablated. In this context, we conclude that hairfans are the sensory organs likely responsible for sound detection in lobsters. While beyond the scope of this study, this could be further confirmed through direct neuronal response measurements to sounds on isolated hairfans (as in Laverack 1962).

Finally, sound detection by external hairbodies is intriguing, in part because these sensory organs are widespread in marine invertebrates (Budelmann 1992). While many recent studies have focused on statocysts, external haircells sound detection greatly broadens the potential scope of marine invertebrate hearing.

Ecological implications

This study has important ecological relevance as we have demonstrated the capacities of lobsters for low frequency sound detection. We notably found that the greatest sensitivity range encompasses the fundamental frequencies (80 – 120 Hz) and intensity levels of their buzzing sounds (Figure 11; Fish et al. 1966; Henninger & Watson 2005; Jézéquel et al. 2018). Taken together, these results strengthen the potential role of buzzing sounds for intraspecific communication.

Most behavioural studies have focused on agonistic encounters in male American lobsters, and have shown that they use chemical and visual signals to communicate dominance status (Karavanich & Atema, 1998; Bruce et al. 2018). In addition, Jézéquel et al. (2020a) recently found that male European lobsters also produce many buzzing sounds during these events. Thus, both male *H. americanus* and *H. gammarus* may use sounds as a threat display to

deter conspecifics, as shown in spiny lobsters (*Panulirus argus*; Mulligan & Fisher 1977) and mantis shrimps (*Hemisquilla californiensis*; Staaterman et al. 2011). Furthermore, male lobsters mostly produce buzzing sounds after the first agonistic encounter between dominant and submissive individuals (i.e. when the dominant status is established; Jézéquel et al. 2020a). It has been shown that chemical signals (i.e. pheromones) released in urine are important for preserving the memory of the outcome between pairs of individuals, post-encounter (Breithaupt & Atema 1993; Karavanich & Atema 1998; Breithaupt et al. 1999). Thus, male lobsters could also produce buzzing sounds to recall the outcome of past encounters, in order to avoid additional fights and lower their risk of injury (Breithaupt & Atema 2000).

We compared the differences of sound detection between males and females. This seems to be the first of such comparisons for marine invertebrates. We did not find any significant differences between both sexes which is not surprising considering their similar anatomical morphology. Female lobsters are known to produce buzzing sounds that have similar features as males (Henninger & Watson 2005). However, the natural acoustic behaviour of female lobsters has not been evaluated. Interestingly, berried (egg carrying) females also use agonistic encounters (like males) towards conspecifics to protect their territory and eggs (Mello et al. 1999). In addition, female and male lobsters display shelter sharing and chemical communication during reproduction (Atema & Engstrom 1971, Cowan & Atema 1990). Both female and male lobsters could thus use buzzing sounds to communicate during these important behaviours. For example, dominant males may produce buzzing sounds to attract females in their shelters for reproduction, as shown in semi-terrestrial crabs (Popper et al. 2001). We focused on adult lobsters in this study, and did not test the hearing sensitivity of juvenile lobsters; neither sound detection or sound production abilities of Juvenile lobsters are yet known.

Difficulties associated with tank acoustics

Although one can easily quantify the acoustic frequencies used in a hearing test, it is much more difficult to assess accurate SPL and PAL sound levels and detection thresholds, especially for experiments in tanks (Akamatsu et al. 2002, Jézéquel et al. 2018). In the present study, the frequencies tested were below the tank resonant frequency (i.e. natural frequencies of vibration due to the structural properties of tank walls), and thus SPLs and PALs attenuate fast when propagating away from the receiver (Jézéquel et al. 2019). To circumvent this issue, one must properly calibrate the received SPLs and PALs received by the animal (e.g., Jones et al. 2019). Here, we performed acoustic calibration at the location of the brain and statocysts because we initially assumed that statocysts were the sensory organs for sound detection, based on the bioacoustic literature (Lovell et al. 2005; Hughes et al. 2014; Radford et al. 2016). However, since lobsters are likely using hairfans along their whole bodies as acoustic receptors (see Section Hearing organ above), calibrations at the sensory organ becomes more challenging. The SPL and PAL values vary along the body axis, and differ from those measured at the head location. Thus, the SPLs and PALs sensed by the animals may be either smaller (e.g. with hairfans on body and legs that were further away from the speaker) or higher (e.g. with hairfans present in the claws, as shown in Laverack 1962, that were closer to the speaker). Measuring at the brain allows for an integration of these signals. However, given these considerations, it is important to remember that the thresholds presented in this study are estimates, and that they are related to the methodology we used.

Using both auditory thresholds and buzzing sound features, one may be tempted to estimate communication distances in lobsters, i.e., the distances at which they can detect sounds. However, it is important to note here that both auditory thresholds (from this study) and the buzzing sound intensities (reported in Fish 1966; Henninger & Watson 2005; Jézéquel et al. 2018) were obtained in small tanks where low frequencies are highly attenuated (Jézéquel

et al. 2019; Jones et al. 2020). As a result, sound levels are difficult to assess, and associated uncertainties are likely important. If these results are used to infer communication distances, one must be particularly careful at properly assessing associated uncertainties. As an alternative, one may perform field experiments with free moving lobsters in their habitat. Assessing the propagation features of their buzzing sounds underwater should permit assessment of communication distances, as has been done recently for spiny lobsters (Jézéquel et al. 2020b).

Conclusion

This study has demonstrated low frequency sound detection by lobsters, with the greatest sensitivity range encompassing the fundamental frequencies (80 – 120 Hz) and the intensity levels of their buzzing sounds. These results imply that this hearing ability could be used in intraspecific communication. This sound-sensitivity also suggests that anthropogenic noise may potentially affect lobsters (NRC 2003). Anthropogenic noise dominates low frequencies (below 1 kHz), overlapping the hearing ranges of many marine animals (Clark et al. 2009), including lobsters. A large body of literature has already shown various impacts in marine mammals, fish and cephalopods, from temporary changes in animal behaviours to lethal impacts (Madsen et al. 2006; Hawkins et al. 2015; Jones et al. 2020). In marked contrast, the potential impacts on crustaceans are still poorly understood (Edmonds et al. 2016). Thus, our results on sound detection by lobsters are a first important step that will help further studies to assess the potential impacts of anthropogenic noise on their behaviours.

Acknowledgements

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Partie 2 : la langouste, comme un grillon dans la mer ?



Préambule

Le choix du second modèle d'étude a été motivé par diverses raisons. Comme pour le homard Européen, les connaissances sur l'écologie et la bioacoustique de la langouste rouge sont faibles comparées à la littérature importante qui est disponible sur les différentes espèces de langoustes tropicales. De plus, cette espèce a un fort intérêt patrimonial, de par son importante histoire dans les pêcheries du milieu du XXème siècle qui se sont développées en Europe. Nous verrons également par la suite que la bioacoustique de la langouste rouge est fondamentalement différente de celle du homard, ce qui en fait donc un modèle d'étude largement différent.

Une espèce victime de la surpêche en Europe

La langouste rouge *Palinurus elephas* (Fabricius, 1787) vit sur les côtes Européennes de l'Atlantique Nord Est à la Méditerranée, et se retrouve depuis la zone infralittorale jusqu'à 200 m de profondeur (Hunter 1999). Sa forte valeur commerciale a entraîné un fort développement de sa pêche durant l'après-guerre (1945-1960) en France. Cette forte pression de pêche, le passage de l'utilisation du casier au filet, ainsi que l'absence de gestion, ont entraîné l'effondrement rapide des stocks de langoustes (Goñi and Latrouite 2005). C'est le cas notamment à Audierne (Bretagne), un des principaux ports de pêche à la langouste en France, où les débarquements chutent de 1000 tonnes en 1950 à moins de 20 tonnes en 2000 (Latrouite and Lazure 2005). Le scénario est classique et aujourd'hui, cette espèce est classée comme vulnérable sur la liste rouge IUCN en Europe (Goñi 2014).

Suite à cet effondrement, des mesures de gestion ont récemment été mises en place par un collectif de pêcheurs associé à Ifremer et au Parc Marin d'Iroise (PMI) en Bretagne. Outre l'augmentation de la taille minimale de capture, une des principales décisions a été d'instaurer en 2007 un cantonnement (zone interdite à la pêche) dans la zone de l'île de Sein, i.e. la zone la plus riche en langoustes de France (PMI 2016). Des suivis de l'évolution des stocks de

langoustes dans cette zone sont depuis effectués annuellement, et consistent à dénombrer des individus en plongée, et à estimer l'abondance à l'aide de capture-marquage-recapture en utilisant des filets (Gervois 2015). Considérant les nombreux défis techniques et éthiques associés à ces deux méthodes de suivi (voir sous section dans l'Introduction Générale), il est crucial de développer de nouveaux outils d'étude pour suivre cette espèce dans son environnement. Ce besoin est d'autant plus d'actualité que le nombre de langoustes juvéniles tend à ré-augmenter sur les côtes Bretonnes depuis 2013 sans que l'on puisse expliquer ce soudain retour (Cotten 2016).

Un mécanisme de production sonore similaire aux insectes

Parmi les crustacés, les langoustes ont été étudiées depuis de nombreuses décennies dans la communauté bioacoustique car elles produisent des sons très caractéristiques (Moulton 1957, Meyer-Rochow and Penrose 1976, Patek et al. 2009). A l'inverse du homard, leur mécanisme de production sonore est situé à l'extérieur de la carapace, et est localisé à la base de chaque antenne secondaire (Figure 15). Cet appareil stridulatoire est constitué de deux parties : le plectrum, strié et souple, frotte d'avant en arrière sur la partie antérieure de la surface dure en forme de lime (« file » en anglais, cf Figure 15) et recouverte de bardeaux microscopiques (Meyer-Rochow and Penrose 1974). Ce mécanisme de production sonore est similaire à la stridulation chez les insectes (d'où le surnom des langoustes de grillon des mers ; Patek 2001). Ce mouvement génère ainsi une impulsion large bande à chaque mouvement. Le son produit pendant un mouvement complet est composé de plusieurs impulsions, et ce train d'impulsions est appelé un rasp d'antennes.

Comme chez le homard Américain, le rôle écologique des rasps d'antennes est supposé être associé au contexte anti-prédateur, servant aux langoustes dépourvues de pinces à se défendre face aux prédateurs (Bouwma and Herrnkind 2009, Staaterman et al. 2010). Cependant, le rôle des sons produits dans une éventuelle communication intraspécifique n'est

pas encore démontré. Par ailleurs, Kikuchi et al. (2015) utilisent l'acoustique passive pour détecter les sons des langoustes japonaises (*P. japonicus*), et mettent en lumière la relation entre production sonore et cycles de marées. Dans ce contexte, il apparaît intéressant d'étudier la production sonore de la langouste rouge (*P. elephas*) afin de statuer sur le potentiel acoustique des suivis in situ par acoustique passive à des fins d'écologie, d'éthologie, voire d'estimation d'abondance de cette espèce.

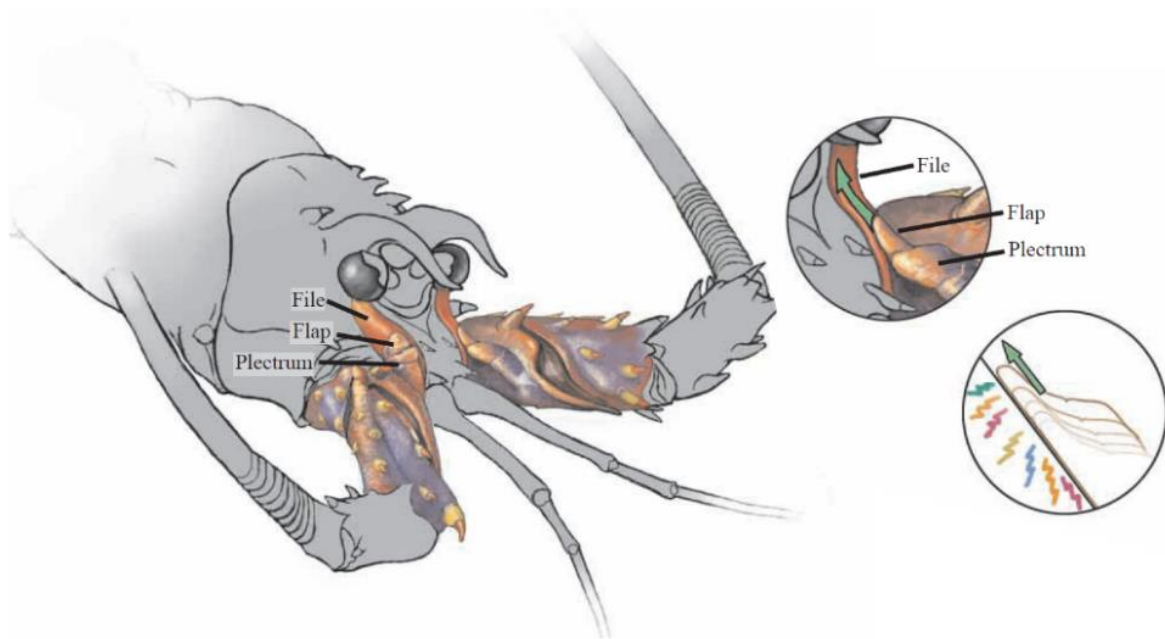


Figure 15: Schéma conceptuel du mécanisme de production sonore chez *P. argus*. Tiré de Patek (2002)

Plan de la partie 2

En nous basant sur les connaissances acquises et présentées dans la littérature bioacoustique centrée sur les langoustes, nous avons effectué deux différentes études complémentaires sur la production sonore de *P. elephas*. Nous proposons de présenter ces travaux selon deux chapitres :

Le **chapitre 4** a pour but de comparer les caractéristiques sonores des rasps d'antennes produits par la langouste rouge en cuve et *in situ*, en continuité des résultats obtenus chez le homard Européen et présentés dans le **Chapitre 1**.

Le **chapitre 5** étudie ensuite la propagation de ces rasps d'antennes dans le milieu marin pour estimer des distances de détection, et démontre le fort potentiel de l'acoustique passive pour suivre cette espèce dans son environnement.

Chapitre 4 : Comparaison des rasps d'antennes de langoustes en cuve et *in situ*



Résumé

Les différentes espèces de langoustes tropicales et tempérées représentent un des groupes de crustacés les plus étudiés dans la littérature bioacoustique. En effet, nous avons rapporté pas moins de 14 articles scientifiques* portant sur la caractérisation de leurs émissions sonores, la première étude datant des années 1950 (voir Annexe 1). Cependant, une lecture attentive de cette littérature fait apparaître, comme pour le homard, que la majeure partie de ces études (13 sur 14 articles répertoriés) se sont attachées à caractériser les rasps d’antennes des langoustes (dont *P. elephas*) en laboratoire uniquement. Nous rappelons que les sons impulsionnels large bande, comme ceux émis par les langoustes, sont fortement déformés par la physique des cuves (i.e. Chapitre 1). Il apparaissait donc opportun de comparer les caractéristiques sonores des rasps d’antennes produits par *P. elephas* en cuve et *in situ*.

Sans surprise, nous avons trouvé des différences significatives dans toutes les caractéristiques sonores (temporelles, énergétiques et fréquentielles) entre les enregistrements sonores des langoustes réalisés en cuve et *in situ*. Nous confirmons ainsi que les rasps d’antennes (et les sons de type large bande globalement) ne peuvent pas être caractérisés sans biais si l’impact physique des cuves sur ces sons n’est pas pris en compte (Jézéquel et al. 2018).

La différence la plus frappante est dans la forme spectrale des rasps d’antennes. En effet, les sons enregistrés en laboratoire étaient à la fois déformés par la résonance dans les hautes fréquences (> 2 kHz), mais aussi par l’atténuation des basses fréquences (< 2 kHz). Le spectre observé est ainsi similaire à ceux précédemment publiés chez cette espèce mais il s’écarte de la réalité mesurée *in situ*. Nous avons donc enregistré à plusieurs reprises et pour la première fois les rasps d’antennes de la langouste rouge dans le milieu marin. Ces sons étaient également large bande, mais présentaient à l’inverse un contenu énergétique majoritairement présent dans les basses fréquences (< 1 kHz).

* Ce nombre paraît dérisoire face aux études de bioacoustique des mammifères marins ou des poissons, mais reste important pour les crustacés. A titre de comparaison en 2017 (début de la thèse), on dénombrait 3 articles sur la bioacoustique du homard américain.

Il convient de noter que caractériser avec précision un son est crucial pour être capable de le détecter dans le milieu marin parmi les autres sources sonores, mais aussi pour en déduire son potentiel rôle écologique. Peu d’études ont émis l’hypothèse d’un rôle écologique des rasps d’antennes produits par les langoustes pour la communication intraspécifique. En effet, ces études réalisées en cuve ont montré que ces sons ne présentent de l’énergie que dans les hautes fréquences (e.g. > 10 kHz ; Buscaïno et al. 2011a), et sont donc supposés ne pas être détectables par les crustacés (Budellman 1992, Popper et al. 2001). Nous expliquons le manque de contenu basse fréquence dans ces enregistrements historiques comme un artefact dû à la propagation sonore en cuve, et non comme une absence de ces fréquences produites. En effet, nos enregistrements *in situ* montrent la dominance du contenu énergétique des rasps d’antennes dans les basses fréquences (< 1 kHz). Nous pouvons donc émettre l’hypothèse du rôle de ces rasps d’antennes pour la communication intraspécifique. Cette production sonore pourrait notamment être utilisée pour menacer des conspécifiques lors de rencontres agonistiques pour l’accès à la nourriture ou l’accès à des refuges (Berrill 1976).

Enfin, et en contraste marqué avec la littérature bioacoustique des langoustes, nous avons pu démontrer pour la première fois que les rasps d’antennes sont très intenses. En effet, durant nos enregistrements, ces sons étaient tous détectables *in situ* malgré un bruit ambiant important. Nous avons ainsi souligné le fort potentiel acoustique de *P. elephas*, dont les sons pourraient être utilisés comme outil de suivi *in situ* par acoustique passive.

Article n°4:

Revisiting the bioacoustics of European spiny lobsters *Palinurus elephas*: comparison of antennal rasps in tanks and *in situ*

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Abstract

Spiny lobsters (Palinuridae) are capable of emitting sounds called antennal rasps. In the bioacoustics literature, such broadband sounds have mostly been characterized from tank recordings where reverberation and resonant frequencies might strongly distort their features. Hence, in this study, we compared antennal rasps produced by European spiny lobsters *Palinurus elephas* in both tank and *in situ* conditions. We found significant differences in all sound features (temporal, intensity and spectral features) between tank and *in situ* recordings, confirming that antennal rasps — and broadband sounds generally — cannot be accurately characterized in tanks if sound reverberation is ignored. In recordings of antennal rasps made *in situ*, we show that the main acoustic power is located in the low frequency band (below 1 kHz), which was missed by all earlier studies done in tanks where such low frequencies cannot be properly measured. The hearing capacities of crustaceans suggest roles for intra-specific communication of these sounds, and their high levels indicate they could be heard above noise. Indeed, we outline that antennal rasps are among the loudest sounds known in the marine animal kingdom, with peak-to-peak sound pressure levels (calculated at 20 cm from the source) above 175.7 dB re 1 μPa^2 , and peak-to-peak source levels (estimated at 1 m from the source) ranging from 154.2 to 160.6 dB re 1 μPa^2 . These acoustic properties imply they could be detected *in situ* during passive acoustic monitoring. This study also highlights the importance of using appropriate measurement methods when characterizing sounds produced by marine invertebrates.

KEY WORDS

Passive acoustics | Spiny lobsters | Palinuridae | *Palinurus elephas* | Antennal rasp | Tank reverberation | Resonant frequencies

Introduction

Ecologists are looking for new environmental descriptors to monitor marine ecosystems. Among the different tools available to survey coastal ecosystems, passive acoustics seems promising, mostly because it is non-invasive and non-destructive (Rountree et al. 2006). Recent studies have highlighted the potential of passive acoustics to monitor tropical marine ecosystems by focusing on producers of specific sounds, such as snapping shrimps and fish (Lammers et al. 2008, Kaplan et al. 2015, Deichmann et al. 2018). In contrast, temperate marine ecosystems have received much less attention, and there is a growing interest to identify species producing sounds that can be detected and isolated among ecosystem soundscapes. In addition to accurately characterizing sounds emitted by these species, we also need to be able to associate them with particular behaviors to understand their ecological meanings (Briffa et al. 2003, Ladich and Myrberg 2006). These steps are crucial before envisaging the use of biological sounds as potential marine ecosystem indicators. Crustaceans should be considered as having high potential for bioacoustics studies because they emit a large diversity of sounds (Staaterman 2016), particularly in temperate coastal waters (Coquereau et al. 2016).

Among marine crustaceans, tropical spiny lobsters have received attention for many decades in marine bioacoustics because they produce specific sounds (Moulton 1957, Meyer-Rochow and Penrose 1976, Patek et al. 2009). The mechanism of sound production is external, is located at the base of each second antenna and consists of 2 parts: the soft, ridged plectrum that rubs posteriorly over the anterior part of the hard file-like surface covered with microscopic shingles (Meyer-Rochow and Penrose 1974, Patek 2001, Patek and Baio 2007). This stick-and-slip movement generates a series of broadband pulses during each slip. The associated sound produced during a full movement is composed of many short pulses (the pulse train), and each pulse train is called an 'antennal rasp' (Moulton 1957, Hazlett and Winn 1962b, Patek et al. 2009). The ecological meaning of antennal rasps has mostly been attributed to an anti-predator

context, such as helping clawless spiny lobsters to deter or escape from predators (Bouwma and Herrnkind 2009, Staaterman et al. 2010). However, far less is known about the bioacoustics of spiny lobsters living in temperate coastal waters (Buscaino et al. 2011a, b).

The European spiny lobster *Palinurus elephas* (Fabricius, 1787) inhabits European coastal waters from the Northeast Atlantic to the Mediterranean Sea (Ceccaldi and Latrouite 2000) and occurs from the shoreline down to about 200 m water depth (Hunter 1999). It is a large, mobile crustacean with an activity pattern characterized by diurnal sheltering and nocturnal foraging (Giacalone et al. 2015). Its life cycle is quite different from other benthic crustaceans, because it has an unusually long pelagic larval stage (between 6 and 12 mo) followed by benthic juvenile and adult stages, with growth happening through successive molts (Hunter 1999). Because of its high commercial value, *P. elephas* has been historically overfished in many European waters (Goñi and Latrouite 2005), which brought this species to its current status of ‘Vulnerable’ on the IUCN Red List (Goñi 2014). A striking example of population collapse is found in Brittany, France, where landings dropped from 1000 t in the 1950s to < 20 t by the 2000s (Goñi and Latrouite 2005). In this context, it is critical to find new study tools, such as passive acoustics, to monitor this species in these areas.

Antennal rasps produced by spiny lobsters, including *P. elephas*, have mostly been investigated and characterized in tank experiments (e.g. Hazlett and Winn 1962a, Mulligan and Fischer 1977, Buscaino et al. 2011a; see Annex 1). These studies in tanks are necessary before performing *in situ* recordings, because they permit isolating the sounds produced by the studied species, without ambiguity from other sources of sounds present in the oceans. Tank studies are also useful to make visual observations that are essential to associate sounds with behaviors. However, sound characterization in tanks presents many challenges, particularly for broadband sounds that are commonly produced by crustaceans. In a previous article, Jézéquel et al. (2018) illustrated that reverberation in small, constrained volumes like the tanks often used in marine

bioacoustics studies, extends the sound duration. In addition, the resonant frequencies, which are specific to each tank’s dimensions (Akamatsu et al. 2002), distort the spectral shape of broadband sounds so that their intensity and spectral features cannot be characterized properly (Jézéquel et al. 2018). Moreover, low-frequency sounds (i.e. with frequency below the first resonant frequency of the tank used) are highly attenuated and cannot be measured properly (Rogers et al. 2016). Thus, broadband sounds, such as antennal rasps produced by spiny lobsters, should not be characterized in tanks without a full understanding of the sound reverberation in the tanks. Ideally, tank recordings should be complemented by *in situ* recordings, which mitigate the reverberation issue, while introducing other experimental difficulties e.g. visibility, ambient noise). Accurately characterizing a sound is important to be able to detect it among the myriad other biological sounds present in marine environments (e.g. Tricas and Boyle 2014, Putland et al. 2017), and is also critical for inferring its potential ecological role.

Here we characterized for the first time the antennal rasps emitted by the European spiny lobster *P. elephas* under *in situ* conditions. We first compared different features of these sounds calculated from both an experimental tank and *in situ*, and then compared them with the existing literature. We assessed the potential ecological roles of these sounds based on our results and determined the potential of such sounds for *in situ* passive acoustic monitoring.

Materials and Methods

Ethical statement

Experiments with European spiny lobsters are not subject to restriction for animal scientific research according to the French legislation and the European Community Council Directive of September 2010 (2010/63/UE). However, we followed the ARRIVE guidelines (Kilkenny et al. 2010) to ensure that all experiments were performed under good conditions. Animals were handled with care during the study, and their health status was checked daily by the authors and the aquariology staff of the public aquarium Océanopolis in Brest, France, where the laboratory experimental work was done. No specimens were harmed during this study and there was no mortality. At the end of the study, all specimens were released back into the environment where they were collected.

Antennal rasp recordings and video

Sounds were recorded using 1 pre-amplified hydrophone (HTI-96-MIN, High Tech), with a sensitivity of -163.7 dB re $1 \mu\text{Pa}$ and a flat response from 2 Hz to 50 kHz. This hydrophone was connected to a Wildlife Acoustics Song Meter (Model SM2) recorder with a gain of 0 dB, and was powered by cell batteries. Recordings were made with a sampling frequency of 48 kHz at a 16-bit resolution. The HTI-96-MIN / Wildlife SM2 recording device had the capacity to record sounds with sound pressure levels in peak to peak up to 185 dB re $1 \mu\text{Pa}$. This is crucial to characterize powerful pulses without clipping the recorded sound (i.e. sound saturation).

Video recordings were made during laboratory and *in situ* experiments using 2 GoPro® HERO3 cameras at a recording rate of 29.97 frames s^{-1} . The videos permitted confirmation of antennal rasp production by the spiny lobsters tested.

Laboratory experiment

Animal collection, characteristics and care

The laboratory work was carried out at the Océanopolis public aquarium in Brest. For these experiments, a total of 13 *Palinurus elephas* juveniles (7 males and 6 females) were used. Specimens had a carapace length (CL), as measured from the anterior tip of the rostrum to the medial point of the posterior carapace margin, between 4.2 and 7 cm. They were collected carefully by hand while scuba diving in the Bay of Brest, at depths of between 15 and 25 m during 2 diving sessions on 18 and 19 July 2018. Only inter-molt individuals with full sets of intact appendages were selected for this study. After capture, they were immediately transferred randomly to 4 glass-sided rectangular holding tanks (0.6 × 0.5 × 0.35 m, length × width × effective height; seawater volume = 0.105 m³), with 2 to 4 individuals per holding tank. Each individual was described (size, sex) and given an identification number.

Holding tanks were continuously supplied with sand-filtered, UV-sterilized seawater pumped from the Bay of Brest. Temperature, salinity and animal conditions were controlled twice a day. During the holding period, temperature varied between 17.08 and 18.15°C, and salinity between 34.84 and 35.05. All animals were fed with fresh pieces of fish (mackerel) and cephalopods (squid) ad libitum. They were kept under a 12:12 h light:dark photoperiod; daylight conditions (from 08:00 to 20:00 h) were simulated using fluorescent light tubes above the holding tanks. Several sections of rigid PVC pipe were provided as shelters in each tank. The bottom of each holding tank was supplied with a layer of sand 5 cm deep to provide a suitable foothold surface for the animals. Animals were acclimatized at least 1 mo in these holding conditions before they were used in experiments.

Experimental set-up and conditions of antennal rasp recordings

For the laboratory recordings, we used a plastic sided rectangular tank (1.13 × 0.73 × 0.5 m; 0.4 m³) which was placed in a quiet room. During the experiments, it was continuously supplied

with the same seawater as the holding tanks, and the bottom was also covered with clean sand. One video camera (same model GoPro® as mentioned above) was placed on the bottom of the tank along the center of the short wall, and a second one was placed 50 cm above the center of the tank, looking down (i.e. plan view). During recordings, the tank was lit with 2 LED strips placed 50 cm above the water surface.

Before antennal rasp recordings, the flow of seawater in the room was cut off. The ambient noise of the experimental tank was recorded for at least 10 min without the animals to check for any additional electrical or other transient sounds. Spectrum analysis of the ambient noise in the experimental tank showed the absence of any peak frequencies compared to those that were present during sound recordings made with animals. At low frequencies (≤ 50 Hz), peaks present corresponded to electrical noise from the recorder. These peaks were excluded from the analyses of biological sounds.

Next, we carefully transferred 1 spiny lobster from its holding tank to the experimental tank and waited for the individual to stop exploring and assume a resting position (about 5 min). The animal was then gently picked up and held at distances of between 20 and 30 cm from the hydrophone and about 20 cm off the substrate to elicit antennal rasps, as is commonly described in the bioacoustics literature on spiny lobsters (Moulton 1957, Meyer-Rochow and Penrose 1976, Patek et al. 2009). Antennal rasp recordings lasted about 30 to 60 s (individuals stopped emitting antennal rasps after this period), and all individuals were tested once during the study. After recordings were complete, each individual was returned to its holding tank, and sound files were archived for analysis.

In situ recordings

Site description

For comparison, we also recorded antennal rasps from spiny lobsters *in situ*, in the Bay of Perros Guirec, Brittany, France, where small groups of juveniles have been observed by recreational divers. Three different spots were selected in the bay, within 3 km of each other, where small groups of individuals were present. Juveniles were observed to be in typical habitats (similar to habitats described in the Mediterranean Sea; Díaz et al. 2001) of vertical, rocky outcrops (approximately 5 to 10 m high), with less than 12 m seawater depth at low tide. They were covered with colonies of jewel anemones (genus *Corynactis*) and colonial gorgonians (*Eunicella verrucosa*) just below the kelp belt (*Laminaria digitata*). These outcrops have many crevices inhabited by juvenile spiny lobsters. Because these areas are often subject to strong water currents (>2 knots), we performed antennal rasp recordings 30 min before and after low tide, when tidal currents were lowest.

In situ recordings of antennal rasps

Antennal rasp recordings were made on 29 September, and again on 21 October 2018 at the sites described above. We deployed the recording device about 2 m from the rock face and at 2 m above the substrate, in front of the crevices where spiny lobsters were visible. First, the ambient noise at each site was recorded for 10 min. Just before sounds were recorded, 2 experienced divers free-dived to explore the vertical rocky outcrops and note the different locations where spiny lobsters were found. This permitted us to observe and determine individually the different spiny lobsters that could be tested, to avoid testing the same lobster twice. After this, we first filmed and then carefully collected an individual spiny lobster and brought it quickly to within 20 to 50 cm of the hydrophone, and 10 m below the seawater surface. During the manipulation, the spiny lobster was held so that it faced the hydrophone, and pointed away from the rocky face. The individual was then gently released back into its

crevice. All individuals that were successfully caught were tested once, and each antennal rasp recording session lasted around 30 to 60 s. Carapace length and sex for each tested individual were both noted. Because the antennal rasp recordings were performed while freediving, background noise from the diving activity was minimal in the vicinity of the recording device.

Sound analysis

Sound features of antennal rasps

Synchronized recordings of sounds (in .wav format) and videos were analyzed to confirm antennal rasp production by the spiny lobsters tested. Based on this annotation, each antennal rasp was extracted manually using the Audacity software® (version 2.1.1; Audacity Team 2015). Antennal rasps were defined as pulse trains composed of several pulses separated by less than 20 ms from each other. Hence any isolated pulses (mostly present in the *in situ* recordings) were not analyzed here. All sequences were then processed using custom MATLAB scripts (version 9.1; The MathWorks). Antennal rasps from tank recordings were analyzed between 60 Hz and 24 kHz to exclude electrical noise; frequencies below 60 Hz were ignored but not filtered. On the other hand, antennal rasps from *in situ* recordings were analyzed between 0 Hz and 24 kHz. No infra-sounds related to electronic self-noise from recording devices used were present during *in situ* recordings.

Antennal rasps from tank and *in situ* recordings were characterized by examining 3 different types of sound features: temporal, intensity and spectral features. The different sound features are presented as mean \pm SD.

For tank and *in situ* recordings, we calculated 3 different temporal features: total duration (in ms), number of pulses per antennal rasp and pulse rate (in Hz). Total duration was calculated as the duration between the first ‘rise’ of the first and the last pulses of each antennal rasp (Jézéquel et al. 2018). This allowed us to avoid some of the effects of reverberation that extended each single pulse duration in the experimental tank. The pulse rate was defined as the

number of pulses per train divided by the total duration. When pulses from an antennal rasp could not be clearly isolated from each other (i.e. because the reverberation of one pulse overlapped with the next one, or because 2 antennal rasps occurred simultaneously), we did not calculate its temporal features.

We also calculated 2 different intensity features based on sound pressure level (SPL, in dB re 1 μPa^2). As antennal rasps are pulse trains characterized by short and transient sounds, we chose to calculate the SPL in peak-to-peak (SPLpp) which is the most representative intensity feature for these types of sounds (Erbe 2010). We also calculated the SPL as a root mean square (SPLrms) that can be applied to pulse trains if they are homogeneous in time and long enough, so that they look like continuous signals at the scale of the analysis window.

In addition, for antennal rasps recorded *in situ*, source levels were estimated as peak-to-peak (SLpp) at 1 m from the spiny lobsters, based on SPLpp and a model of transmission loss (TL). As the hydrophone was located near the sound source (i.e. <1 m from the spiny lobsters), we assumed the antennal rasps propagate uniformly in all directions, permitting the use of a simple spherical spreading loss model to account for TL (Erbe 2010). Thus, SLpp was estimated as follows: $\text{SLpp} = \text{SPLpp} + \text{TL}$, with $\text{TL} = 20\log_{10}(r)$ where r indicates the distance (in meters) between the spiny lobster and the hydrophone. Because it was difficult to maintain spiny lobsters underwater at a constant distance from the hydrophone during antennal rasp recordings while free-diving, we estimated the r from the videos as a minimum and maximum distance between the individual tested and the hydrophone. We estimated these distances between the spiny lobster held by the first diver and the hydrophone through the video recordings done by the second diver, and by knowing the dimensions of the recording device. The second diver filmed perpendicular to the direction of the spiny lobster held near the hydrophone, so that the Pythagorean theorem could be applied to calculate these distances. We then obtained 2 different peak to peak source levels: SLpp min and SLpp max. Note that in this study, the *in situ* SLpp

estimates ignored any near-field effect. Because the use of more complex models of TL should be applied for antennal rasps recorded in the experimental tank, and because those models require a very accurate estimation of the source–receiver distance (Rogers et al. 2016), we did not estimate their SL_{pp}.

We also calculated 3 different spectral features: The first and second peak frequencies (Fp1 and Fp2, respectively; in kHz) represent the 2 frequencies where the power spectral density (PSD, in dB re 1 $\mu\text{Pa}^2 \text{Hz}^{-1}$) was maximal. These frequencies for antennal rasps recorded in the experimental rectangular plastic tank were compared with the minimum resonant frequency of the tank. This minimum resonant frequency (in kHz) was calculated using the theoretical equation from Akamatsu et al. (2002) for a rectangular glass-sided tank with mode (1, 1, 1). Finally, the frequency bandwidth (B , in kHz) was estimated as the spread (i.e. standard deviation) of the PSD around Fp1.

Ambient noise characterization

Recordings of ambient noise (10 min each) from the 5 *in situ* spots were first visualized to ensure the absence of antennal rasps. Next, they were both cut into 10 sequences of 1 min each, and we randomly selected 3 of 10 sequences from each recording per site. Ambient noise is characterized as a continuous sound (Erbe 2010); we thus calculated the SPL_{rms} of each selected, 1 min long sequence. This provided a mean value of the SPL_{rms} for the ambient noise at each spot. We also generated the sets of theoretical Wenz curves (Wenz 1962) for boat traffic noise (ranging from index 1 to 7) and wind noise (ranging from 0 to 30 knots) using custom MATLAB scripts. They were used as a comparison for the PSDs of the ambient noise calculated at each spot.

Statistical analysis

We first tested whether differences in means between the carapace length of individuals tested between the 2 groups (tank and *in situ*) were significant. As these data were normally distributed

Partie 2 – Chapitre 4 : Comparaison des rasps d'antennes de langoustes en cuve et *in situ*

in both groups (Shapiro-Wilk test, $p < 0.05$), Student's *t*-test was applied ($\alpha = 0.05$). All sound features described above (except for the SLpp that was only calculated for antennal rasps recorded *in situ*) were then tested to determine whether significant differences in means were evident between the 2 groups. We aimed to compare tank and *in situ* recordings of antennal rasps produced by *P. elephas* using the same handling protocol. Thus, no variability among antennal rasps produced by a single individual, nor between individuals could be considered from the data collected. Considering the small number of sound recordings, and assuming that calculated variables for each individual can be assimilated to a random distribution, the non-parametric Mann-Whitney *U*-test was used to determine if their probability distributions were equal ($\alpha = 0.001$). All statistical analyses were performed using R version 3.5.1 (R Core Team 2018).

Results

Sound features of antennal rasps

In both tank and *in situ* recordings, all spiny lobsters tested produced audible antennal rasps during episodes of stress generated while handling. These were mostly accompanied by visible, vigorous contractions of their abdominal regions.

Tank recordings

A total of 387 antennal rasps from the 13 individuals tested (21–34 rasps ind.⁻¹) were recorded in the experimental tank. Waveforms consisted of pulse trains that were sometimes difficult to isolate because of sound reverberation (Figure 16A). The effects of sound reverberation were also seen in spectrograms where all pulses (dark vertical lines) were followed by a ‘smear’ (dark horizontal lines; Figure 16C). When taking this into account, temporal features could be calculated in 94% of the antennal rasp events. Their mean total duration was 120.5 ± 25.9 ms (range: 60–225 ms), with a mean of 15.0 ± 3.3 pulses per antennal rasp (range: 7–28) and a mean pulse rate of 127.9 ± 21.1 Hz (range: 78.4–226.7 Hz) (Table 7).

Intensity features were high, with a mean SPL_{pp} of 171.0 ± 3.1 dB re $1 \mu\text{Pa}^2$, including some antennal rasps recorded at 20 cm from the spiny lobsters of up to 175.7 dB re $1 \mu\text{Pa}^2$ (Table 7). The mean SPL_{rms} was 151.2 ± 4.2 dB re $1 \mu\text{Pa}^2$ (Table 7).

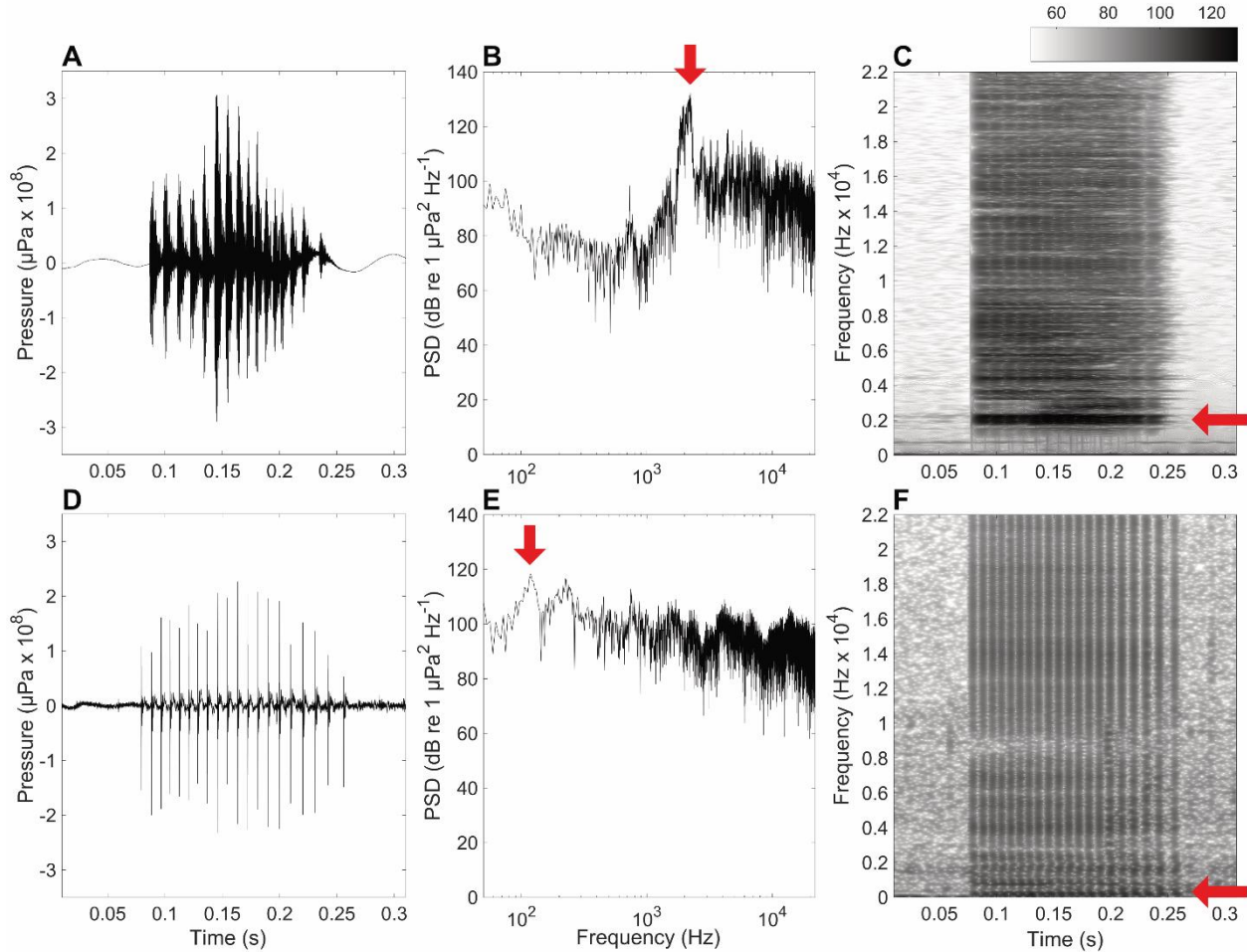


Figure 16: Examples of typical antennal rasps produced by 2 *Palinurus elephas* juveniles and recorded at the same distance from the hydrophone (20 cm) in the experimental tank (top row) and *in situ* (bottom row). (A,D) Waveforms. (B,E) Acoustic spectra (Fast Fourier Transform length: 13640), with the x-axis in logarithmic scale. (C,F) Spectrograms (Fast Fourier Transform length: 2024; Hamming window: 1001 points; 99% overlap). Red arrows indicate the first peak frequency of each antennal rasp. Notice that the first peak frequency of the antennal rasp recorded in the tank (top row) corresponds to the minimum resonant frequency calculated for the experimental tank alone (2 kHz); there is also a large gap of acoustic power below 2 kHz. The color scale bar is in dB re 1 $\mu\text{Pa}^2 \text{Hz}^{-1}$. PSD: power spectral density

Table 7: Sound features (temporal, intensity and spectral features) calculated for the European spiny lobster antennal rasps recorded in the experimental tank and *in situ*. Results are presented as means \pm SD with ranges (minimum–maximum). A Mann-Whitney test was used to compare means of sound features between the tank and *in situ* recordings. **Bold** values highlight sound features that were significantly different (*U*-test, $p < 0.001$) between tank and *in situ* recordings. SPL_{pp} (SPL_{rms}): peak-to-peak (root mean square) sound pressure level, SL_{pp}: peak-to-peak source level, Fp1 (Fp2): first (second) peak frequency, *B*: frequency bandwidth, NA: not applicable

Sound features	Antennal rasps	
	Tank	<i>In situ</i>
Temporal		
Total duration (ms)	120.5 \pm 26.0 (60–225)	147.0 \pm 29.7 (53–266)
Number of pulses per antennal rasp	15.0 \pm 3.3 (7–28)	16.9 \pm 4.7 (6–33)
Pulse rate (Hz)	127.9 \pm 21.1 (78.4–226.7)	115.9 \pm 27.2 (59.4–208.9)
Intensity		
SPL _{pp} (dB re 1 μ Pa ²)	171.0 \pm 3.1 (160.4–175.7)	167.3 \pm 3.9 (156.0–175.7)
SPL _{rms} (dB re 1 μ Pa ²)	151.2 \pm 4.2 (139.7–159.6)	139.2 \pm 3.0 (132.0–146.4)
SL _{pp} min (dB re 1 μ Pa ²)	NA	154.2 \pm 4.5 (142.0–165.3)
SL _{pp} max (dB re 1 μ Pa ²)	NA	160.6 \pm 4.4 (150.0–169.7)
Spectral		
Fp1 (kHz)	3.99 \pm 3.68 (1.82–17.74)	0.77 \pm 0.24 (0.12–1.66)
Fp2 (kHz)	5.34 \pm 4.27 (1.82–17.83)	0.96 \pm 0.40 (0.22–1.62)
<i>B</i> (kHz)	5.13 \pm 2.51 (0.42–11.70)	16.99 \pm 5.38 (4.90–23.00)

The minimum resonant frequency calculated for the experimental tank was 1.94 kHz, and strongly influenced the spectral shape of the antennal rasps. Indeed, 268 out of the 387 (69%) antennal rasps had their first peak frequencies at 2 kHz, which was clearly seen in both the PSDs (Figure 16B) and spectrograms (dark horizontal line at 2 kHz; Figure 16C). Below 2 kHz, a relatively large acoustic power gap was found in all antennal rasps (Figure 16B, C). In contrast, above 2 kHz, high acoustic power was present up to 23 kHz (Figure 16B, C). These higher frequencies corresponded to other resonant frequencies associated with the experimental tank and caused a highly variable F_{p1} and F_{p2} , with means of 3.99 ± 3.68 kHz (range: 1.82–17.74 kHz) and 5.34 ± 4.27 kHz (1.82–17.83 kHz), respectively (Table 7). Because acoustic power was mainly focused around the minimum resonant frequency of the tank, the resulting bandwidth was limited, with a mean of 5.13 ± 2.51 kHz (0.42–11.70 kHz) (Table 7).

In situ recordings

During *in situ* recordings, 9 spiny lobsters (6 females, 3 males) were successfully caught and their antennal rasps recorded in the 3 different spots described above. Their mean CL was 5.1 ± 0.4 cm (SD) and did not differ significantly from the mean CL of spiny lobsters used for the laboratory experiments (*t*-test, $p = 0.32$). A total of 233 antennal rasps were recorded from all 9 spiny lobsters (range: 17–32 rasps ind.⁻¹).

Compared to antennal rasps recorded in the experimental tank, antennal rasps recorded *in situ* presented clear waveforms with pulses that could be easily isolated (Figure 16D). This was also highlighted in the spectrograms, which did not have the smearing seen in the tank recordings (Figure 16C, F). Thus, temporal features could be calculated for all 233 recorded antennal rasps emitted by the 9 individuals tested. Mean total duration was 147.0 ± 29.7 ms (53–266 ms), with a mean number of pulses per antennal rasp of 16.9 ± 4.7 (6–33) and a mean pulse rate of 115.9 ± 27.2 Hz (59.4–208.9 Hz; Table 7). Mean temporal features from antennal

rasps recorded *in situ* were all significantly different from the antennal rasps recorded in the tank; the mean total duration and mean number of pulses per antennal rasp were higher (*U*-test, $p < 0.001$), whereas the mean pulse rate was lower (*U*-test, $p < 0.001$).

As seen in Table 7, even if some SPLpp and SPLrms values were also high (up to 175.7 and 146.4 dB re 1 μPa^2 at 20 cm from the spiny lobsters, respectively), their mean values were significantly lower than those measured in the experimental tank (*U*-test, $p < 0.001$). The mean values of SLpp estimated at 1 m from the spiny lobsters in the field ranged between 154.2 ± 4.5 (SLpp min) and 160.6 ± 4.4 (SLpp max) dB re 1 μPa^2 (Table 7).

As no effects of reverberation were detected for the antennal rasps recorded *in situ*, we could calculate their natural spectral features for the first time. Acoustic power peaks were found to be spread in the low frequency band (below 1 kHz; Figure 16E, F). Indeed, mean values of Fp1 and Fp2 were respectively 0.77 ± 0.24 kHz (0.12–1.66 kHz) and 0.96 ± 0.40 kHz (0.22–1.62 kHz) (Table 7). These were both significantly lower than the tank recordings (*U*-test, $p < 0.001$). This is the contrary to what was seen in the tank recordings where acoustic power was only found from 2 kHz (Figure 16B, C). However, these low peak frequencies did not include all the acoustic power contained in antennal rasps, with some acoustic power also found in higher frequencies (up to 23 kHz). This gave a mean bandwidth of 16.99 ± 5.38 kHz (4.90–23.00 kHz; Table 7) that was significantly larger than that of the tank recordings (*U*-test, $p < 0.001$).

Comparison of ambient noise and antennal rasps recorded *in situ*

During *in situ* recordings, the sea state was between 1 (Calm) and 2 (Smooth) on the Douglas scale, corresponding to wave heights of between 0 and 0.5 m. Wind state ranged between 0 (Calm) and 4 (Light breeze) on the Beaufort scale, corresponding to speeds between 1 and 18 knots. Seawater temperature was $16.5 \pm 0.2^\circ\text{C}$ and salinity was 35.4 ± 0.1 .

Two different groups of ambient noise were distinguished among the 5 different spots where sound recordings were made. Two spots were directly subject to more energetic sea conditions (high wind speeds and waves), resulting in mean SPLrms values of 121.0 ± 0.5 and 124.2 ± 1.0 dB re $1 \mu\text{Pa}^2$. In contrast, the 3 other locations were calmer, which was reflected in a lower mean SPLrms of 109.5 ± 1.6 dB re $1 \mu\text{Pa}^2$. During ambient noise recordings, no antennal rasps were detected.

The PSD of the loudest ambient noise had its main acoustic power peaks at more than 100 dB re $1 \mu\text{Pa}^2 \text{Hz}^{-1}$ below 25 Hz that was attributed to the friction of the hydrophone with water due to the waves (Figure 17). The ambient noise levels at all 5 locations were within Wenz curves of between 25 Hz and 2 kHz. Above 2 kHz, acoustic power peaks were always found around 3 and 4 kHz that were almost 20 dB re $1 \mu\text{Pa}^2 \text{Hz}^{-1}$ above Wenz curves (Figure 17). This contribution was associated with isolated broadband pulses from unknown sources (no snapping shrimps were observed at the spots during the recordings). Despite these observations, PSDs of all antennal rasps recorded at the 5 spots were always above the PSDs of the ambient noise recorded at the same time and over the entire frequency range (except at frequencies below 25 Hz). For example, the PSD of a typical antennal rasp at its first peak frequency (0.125 kHz) was 30 dB re $1 \mu\text{Pa}^2 \text{Hz}^{-1}$ above the PSD of the loudest ambient noise, and almost 50 dB re $1 \mu\text{Pa}^2 \text{Hz}^{-1}$ above the quietest (Figure 17). This pattern was also seen in the higher frequencies up to 23 kHz (Figure 17).

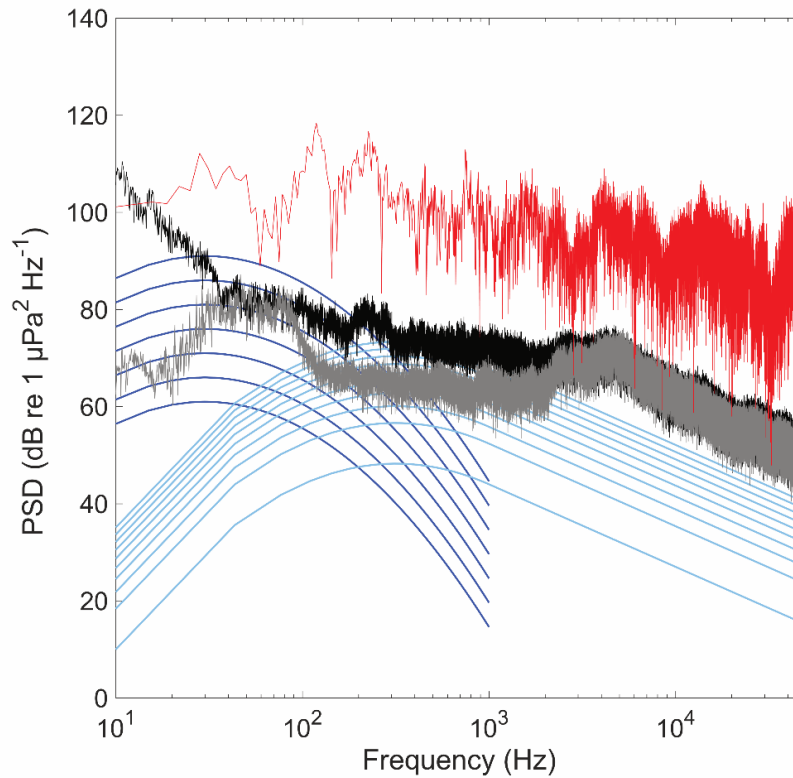


Figure 17: Acoustic spectrum of a *Palinurus elephas* antennal rasp recorded in situ (red), with the loudest ambient noise (black) and the quietest ambient noise (grey) recorded from the 5 different spots. The x-axis is in logarithmic scale. Thin lines indicate Wenz's traffic noise curves ranging from index 1 to 7 (dark blue) and Wenz's wind noise curves ranging from 0 to 30 knots (light blue). PSD: power spectral density

Discussion

In this study of *Palinurus elephas* antennal rasps, we emphasize 2 important results. First, all sound features calculated between antennal rasps recorded in an experimental tank and *in situ* were significantly different. Secondly, this is the first time that *P. elephas* antennal rasps have been characterized *in situ*. We have shown that they are very loud, with SPLpp values (calculated at 20 cm from the source) being higher than 175.7 dB re 1 μPa^2 and SLpp values (estimated at 1 m from the source) ranging from 154.2 to 160.6 dB re 1 μPa^2 , and with important acoustic power spread in low frequencies (below 1 kHz). These new results suggest possible ecological roles for these sounds and a strong potential for them to be detected *in situ*.

Temporal features of the antennal rasps

Sound reverberation in the experimental tank did affect the waveform of antennal rasps by extending the duration of single pulses (Figure 16A, C). This phenomenon was absent in antennal rasps recorded *in situ* (Figure 16D, F). Our results for temporal features are consistent with antennal rasps previously reported for *P. elephas* in the bioacoustics literature. Our mean total durations of 120.5 and 153.1 ms (tank and *in situ* recordings, respectively) were within an order of magnitude of other studies (Patek and Oakley 2003: 101 ms; Buscaino et al. 2011a: 90 ms; de Vincenzi et al. 2015: 70 ms), and are similar to antennal rasp total durations previously published for tropical spiny lobsters (Mulligan and Fischer 1977: *Panulirus argus*, 154 ms; Patek and Oakley 2003: *Panulirus japonicus*, 155.1 ms). In our study, the mean numbers of pulses per antennal rasp recorded in the experimental tank and *in situ* are in the same range as reported for studies done on *P. elephas* (Patek and Oakley 2003: 13.5; Buscaino et al. 2011a: 9.5; de Vincenzi et al. 2015: 8) and other tropical spiny lobsters (Patek and Oakley 2003: *Linuparus trigonus*, 17.9; Kikuchi et al. 2015: *Panulirus japonicus*, 11). Finally, our mean pulse rates of antennal rasps recorded in the experimental tank and *in situ* are close to other studies

Partie 2 – Chapitre 4 : Comparaison des rasps d’antennes de langoustes en cuve et *in situ* on *P. elephas* (Patek and Oakley 2003: 138.5 Hz; Buscaino et al. 2011a: 118.2 Hz; de Vincenzi et al. 2015: 134 Hz), and other species of tropical spiny lobsters (Patek and Oakley 2003: *L. trigonus*, 118.5 Hz; *Justitia japonica*, 131.4 Hz; *Panulirus homarus*, 120.6 Hz).

Even though we recorded and compared 2 groups of juveniles with similar sizes by using the same protocol, we found significant differences in all temporal features between tank and *in situ* recordings. This was surprising, as the temporal features we calculated are not impacted by tank reverberation. This variability could be attributed to differences between individuals and also between antennal rasps produced by the same individual or even the behavioral context (handling); however, these were not investigated for this explanatory study. For example, long legged spiny lobsters *Panulirus longipes* show fatigue after producing antennal rasps for more than 30 s (Meyer-Rochow and Penrose 1976). Fatigue or exhaustion would be expected to lower the rates of antennal rasps and change their temporal features (e.g. pulse rate reduced; Meyer-Rochow and Penrose 1976).

Several authors working on tropical spiny lobsters have examined the scaling of antennal rasp temporal features with body size and even the mechanism of sound production. For example, Patek and Oakley (2003) found positive correlations between pulse rate and the number of pulses with file length, while antennal rasp total durations were negatively correlated with file length for *Panulirus argus*. Meyer-Rochow and Penrose (1976) and Patek et al. (2009) found a positive correlation between body size and antennal rasp total durations, while there was a negative correlation between size and pulse rate for *Panulirus longipes* and *Panulirus interruptus*, respectively. In this study, we used only similar-sized individuals. Performing additional sound recordings using a wider size range could permit verification of antennal rasp temporal features and the importance of inter-individual variability.

Intensity of antennal rasps

This is the first time that such high SPL values for antennal rasps have been reported in the bioacoustics literature on spiny lobsters. We calculated antennal rasp SPL_{pp} values at more than 175.7 dB re 1 μPa^2 in the tank and *in situ* at 20 cm from the specimens tested. In addition, because we recorded antennal rasps *in situ*, we could estimate for the first time their SL_{pp}. We found the SL_{pp} estimated at 1 m from the spiny lobsters ranged between 154.2 and 160.6 dB re 1 μPa^2 . However, 2 different intensity features (SPL_{pp} and SPL_{rms}) were significantly lower in the *in situ* recordings compared to tank recordings. For example, the mean SPL_{rms} of antennal rasps *in situ* was 10 dB re 1 μPa^2 lower than in the tank recordings. This difference in SPL_{rms} is explained by the ‘smear of noise’ accompanying each pulse in all antennal rasps recorded in the tank that increased sound intensity (see Figure 16A, C), and this phenomenon was absent in antennal rasps recorded *in situ* (see Figure 16D, F). In addition, due to the low-frequency sampling of our recording device (48 kHz), these intensity features might be underestimated if there is acoustic power spread at higher frequencies.

Several articles have reported SPLs in decibels for spiny lobsters (Latha et al. 2005, Patek et al. 2009, Buscaino et al. 2011a, de Vincenzi et al. 2015); the numerical values in the literature range from 50 to 150. Unfortunately, these results are not comparable because they were obtained with different signal processing methods, and these earlier studies do not provide enough information to be able to re-estimate the values. For example, calculating an SPL on a frequency representation produces different numerical results depending on the method applied (e.g. power spectrum or PSD) and on the calculation parameters (e.g. sampling frequency, Fast Fourier Transform length). It should also be noted that antennal rasps are short, transient sounds. Fourier-based methods are poorly adapted for such signals, for the reasons previously discussed. As stated in ANSI (2005) and demonstrated by Erbe (2010), it is preferable to use

time-domain metrics such as the SPL_{pp} and SPL_{rms} calculated in our study. Thus, here we chose to compare our results with other studies that also reported intensity features in SPL and SL from sounds emitted by other marine species.

Few marine animals have been reported to emit such loud sounds. Some marine mammals, such as dolphin and whale species, have been shown to produce whistles and calls with SL_{pp} at 1 m that exceed 169 and 189 dB re 1 μPa^2 , respectively (Janik 2000, Sirovic et al. 2007). Some fish have also been shown to emit very loud sounds, including large goliath groupers *Epinephelus itajara* with a maximum SPL_{rms} of 144 dB re 1 μPa^2 (distances from the fish not estimated; Mann et al. 2009), or black drums *Pogonias cromis* with highest SPL_{rms} calculated at 0.95 m exceeding 166 dB re 1 μPa^2 (Locascio and Mann 2011). In crustaceans, only snapping shrimps *Alpheus heterochaelis* have been shown to produce louder impulsive sounds (the 'snaps') compared to the antennal rasps of *P. elephas*. Snaps have been calculated to have SL_{pp} estimated at 1 m ranging from 145 to up to 215 dB re 1 μPa^2 (Cato and Bell 1992, Au and Banks 1998, Schmitz 2002). In the present study, we only recorded antennal rasps in juveniles (CL between 4 and 7 cm); larger adults (CL > 15 cm) may emit even louder antennal rasps if the earlier results of Meyer Rochow and Penrose (1976) for the evolution of antennal rasp features with body size in *Panulirus longipes* can be generalized.

Spectral features

In our study, spectral features of antennal rasps recorded in the experimental tank were strongly affected by the tank resonant frequencies. Indeed, acoustic power was only present from 2 kHz (the minimum resonant frequency of our tank) to 23 kHz. This distribution of acoustic power toward high frequencies in our recordings is generally consistent with the bioacoustics literature on spiny lobsters. Buscaino et al. (2011a) and de Vincenzi et al. (2015) calculated both very high and variable first peak frequencies of 19.52 ± 6.70 and 22.93 ± 8.20 kHz, respectively, for

P. elephas antennal rasps recorded in tanks. Other studies (also done in tanks) for species of tropical spiny lobsters also found high peak frequencies in antennal rasps (e.g. Hazlett and Winn 1962a, Mulligan and Fischer 1977, Patek et al. 2009; see Annex 1). For example, antennal rasps produced by *Panulirus argus* in a tank of similar size to the tank used in the present study showed their first peak frequencies between 2 and 5.5 kHz (Mulligan and Fischer (1977), which should have been described as being associated with the tank resonant frequencies (Akamatsu et al. 2002, Jézéquel et al. 2018). Meyer-Rochow and Penrose (1976) were the first authors to mention the technical challenges of characterizing antennal rasps in tanks because of sound reverberation and resonant frequencies. They reported that antennal rasps were distorted by these phenomena, hence they did not calculate spectral features of the sounds (peak frequencies and bandwidth). These authors concluded that antennal rasps should contain acoustic power spread equally over their bandwidth (i.e. a white spectrum; Meyer-Rochow and Penrose 1976). In contrast, below 2 kHz, we found a large acoustic power gap that was present in all antennal rasps recorded in the experimental tank (Figure 16B, C). This gap below the minimum resonant frequency of our experimental tank is explained by the high attenuation of low frequencies in such tanks because of their longer wavelength compared to the dimensions of the tank used (Rogers et al. 2016).

Not surprisingly, spectral features of antennal rasps recorded *in situ* showed significantly different patterns compared to antennal rasps recorded in the experimental tank. Indeed, the important acoustic power was present in the low frequency band (below 1 kHz; Table 7). These results are representative of the true antennal rasp sound, as our *in situ* measurements were not contaminated by tank reverberation. Thus, our results do not confirm the hypothesis of a white spectrum postulated by Meyer-Rochow and Penrose (1976). However, our results are consistent with the later work of Patek et al. (2009) using *Panulirus interruptus*, where these authors reported the Fp1 in antennal rasps recorded *in situ* as 0.63 ± 0.37 kHz. In

addition, Moulton (1957) described antennal rasps from *Panulirus argus* with peak frequencies of 0.80 kHz in a large naturalized aquarium. However, Kikuchi et al. (2015) recorded antennal rasps *in situ* from *Panulirus japonicus*, with mean values of peak frequencies of 10.00 ± 4.50 kHz. While we used the same protocol as in the existing bioacoustics literature in spiny lobsters to have comparable results, we did not take into account the potential effects of animal handling in the spectral features of antennal rasps. Further studies will be required to characterize antennal rasps without handling animals *in situ*. An interesting perspective is the use of fake predators, as described by Staaterman et al. (2010).

In addition, the bandwidths of our antennal rasps recorded *in situ* were significantly broader compared to antennal rasps recorded in the tank (Table 7). This is due to the minimum resonant frequency of the tank that concentrated the acoustic power, leading to distinct peaks in the PSDs (Figure 16B). In contrast, spectra of antennal rasps recorded *in situ* had acoustic power spread much wider around peak frequencies (Figure 16E). However, the bandwidth values reported here were limited by our system capacity. Because of the sampling frequency (48 kHz), we could not record frequencies higher than 24 kHz. Indeed, the estimated bandwidth (up to 23 kHz) actually covered the whole bandwidth of the recording device, and thus we concluded that the source bandwidth might be greater than 23 kHz. As a confirmation, Buscaino et al. (2011a) found acoustic power up to 100 kHz in antennal rasps produced by *P. elephas* in tanks.

Towards a new biological hypothesis concerning the potential ecological roles of antennal rasps in *P. elephas*

Accurately characterizing a sound produced by a particular species is not only crucial to be able to detect it *in situ* among other biological sounds, but also for inferring biological hypotheses about their potential ecological roles. In the next sections, we discuss the implications of our results.

Inter-specific communication

Most studies in the bioacoustics literature have proposed an anti-predator role for the antennal rasps (Moulton 1957, Meyer-Rochow and Penrose 1976, Staaterman et al. 2010). In this study, we elicited the production of antennal rasps in *P. elephas* by gently handling individuals, which is intended to imitate capture by a natural predator. In contrast with the clawed lobster *Homarus gammarus*, the clawless *P. elephas* relies on its spiny carapace and rigid antennas to defend itself against predators (Barshaw et al. 2003). Thus, being able to emit loud sounds could help individuals to deter potential predators. In contrast with previous studies (Buscaino et al. 2011a, de Vincenzi et al. 2015), we have demonstrated that most of the acoustic power contained in antennal rasps was spread into the low frequency band below 1 kHz. Interestingly, the sound sensitivity of some of their natural predators, namely cephalopods (including octopus), appears to be limited to the same band of low frequencies (Williamson 1988, Packard et al. 1990, Mooney et al. 2010). For example, a controlled experiment with *Panulirus argus* showed that stridulating individuals escaped more frequently from attacking octopuses than surgically muted individuals (Bouwma and Herrnkind 2009). In addition, Buscaino et al. (2011a) found that *P. elephas* produced more antennal rasps when exposed to an octopus than in the trials without an octopus in a tank. As the common octopus *Octopus vulgaris* is a well-known predator of the European spiny lobster (Barshaw et al. 2003) and is typically found in Brittany coastal waters in the same areas inhabited by *P. elephas* juveniles, these antennal rasps may indeed serve as a deterrent.

Other taxa could also be affected by this sound. Several fish species have been described as predators of tropical spiny lobsters, including triggerfish of the genus *Balistes* (Briones-Fourzán et al. 2006, Lavalli and Herrnkind 2009). The bioacoustics literature has shown that most fish hear best below 1 kHz (Ladich and Popper 2004). Thus, even though the triggerfish audiogram has not been described yet, it is likely that it could also hear in this low frequency

band. Bouwma (2006) suggested that antennal rasps produced by *Panulirus argus* against triggerfish is aposematic, advertising the lobster's spiny defenses to predators. Interestingly, the tropical gray triggerfish *B. capriscus* is now a common species in Brittany coastal waters (Quéro et al. 2008) and would encounter *P. elephas*.

Finally, and by examining the spectral features of antennal rasps recorded *in situ*, the large bandwidth (mean of 16.99 kHz) can provide a clear evolutionary advantage in clawless spiny lobsters. It seems possible that those types of broadband sounds could have been selected because they permit their emitters to target a maximum of different potential receivers (i.e. potential predators). Even molted spiny lobsters with a soft carapace can still produce antennal rasps, an observation which has also been reported for the buzzing sounds produced by the European lobster *H. gammarus* (Jézéquel et al. 2018). Thus, even when their protective carapace is weakened, the antennal rasps could still function as a deterrent (Patek 2001).

Intra-specific communication

Few studies have hypothesized a potential intraspecific communication of antennal rasps in spiny lobsters. This could be explained by our observation that most bioacoustics studies have characterized antennal rasps in tanks and reported that acoustic power is only present in high frequencies (above 10 kHz; e.g. Buscaino et al. 2011a), which could not be detected by crustaceans (Goodall et al. 1990, Popper et al. 2001, Lovell et al. 2005). Our findings now allow us to suggest how these sounds could be used as a means of intra-specific communication.

Crustaceans lack gas-filled organs, like swim bladders, required for pressure detection, but may be still capable of detecting low-frequency acoustic stimuli arising from the second component of acoustic energy, i.e. particle motion (Breithaupt and Tautz 1990, Popper et al. 2001, Popper and Hawkins 2018). For example, the Norway lobster *Nephrops norvegicus* responds *in situ* to particle motion over a frequency range of 20–200 Hz (Goodall et al. 1990).

In addition, Lovell et al. (2005) found that common prawns *Palaemon serratus* in tanks are sensitive to particle motion generated by low-frequency sounds ranging from 100 Hz to 3 kHz. Diverse sensory receptors, adapted to detect particle motion from low-frequency sounds, have been described in crustaceans, including statocysts and sensory hairs (Popper et al. 2001, Radford et al. 2016). In this study, we did not measure the particle motion arising from antennal rasps *in situ*. However, as the pressure component associated with antennal rasps is very high, it is likely that the associated particle motion would also be elevated (Nedelec et al. 2016). Additional studies are now required to measure and understand the potential directionality of particle motion/sound generated by these antennal rasps *in situ* (Popper and Hawkins 2018). We also need to quantify the detection bandwidth and thresholds (i.e. audiogram) in *P. elephas* through other means, such as behavioral studies (Goodall et al. 1990).

Spiny lobsters, and particularly juveniles such as those used in this study, are gregarious and generally live in high densities in shelters (Atema and Cobb 1980). Several studies have found that they use chemical and visual signals to maintain a social status inside a group of conspecifics through the use of aggressive agonistic encounters (Zimmer-Faust and Spanier 1987, Ratchford and Eggleston 1998, Shabani et al. 2009). Our results imply that antennal rasps may also be involved. Interestingly, Berrill (1976) showed that post-puerulus larvae of *Panulirus longipes* use agonistic encounters when competing for limited access to food and shelter, and that they use antennal rasps during these stressful events as a threat display.

Antennal rasp detection in underwater soundscapes

In contradiction with the existing bioacoustics literature, our study has highlighted the high acoustic potential of antennal rasps produced by spiny lobsters *in situ*. Our results clearly suggest that antennal rasps produced by *P. elephas* could be detected *in situ* above the ambient noise. Surprisingly, earlier studies carried out with tropical spiny lobsters *Panulirus interruptus*,

using the same protocol of sound recordings as in this study, stated that antennal rasps recorded *in situ* are as loud as the ambient noise, so that they could not be detected (Patek et al. 2009, Staaterman et al. 2010). As discussed in Section 4.2, because the use of metrics to characterize sounds is not normalized (and sometimes missed), it is not possible to do a direct comparison of our results with those of Patek et al. (2009). Nonetheless, our ambient noise measurements (PSD, in dB re 1 uPa² Hz⁻¹) are fully consistent with other measurements performed in the same area (Mathias et al. 2016, Kinda et al. 2017).

Conversely, our hypothesis of antennal rasp detection *in situ* is consistent with the work of Kikuchi et al. (2015) who recorded *in situ* antennal rasps from *Panulirus japonicus*. These authors found a positive correlation between the frequency of detected antennal rasps and the number of spiny lobsters caught in nets, and also showed an increase in detected antennal rasps during night time. The European spiny lobster *P. elephas*, similar to *Panulirus japonicus*, has an activity pattern characterized by diurnal sheltering and nocturnal foraging (Giacalone et al. 2015). During nocturnal movements, *P. elephas* individuals could encounter other conspecifics or predators, leading to the production of antennal rasps that would be detectable with hydrophones. However, further studies are needed to validate this potential method of detection and the distance of sound propagation at potential monitoring locations with high lobster densities.

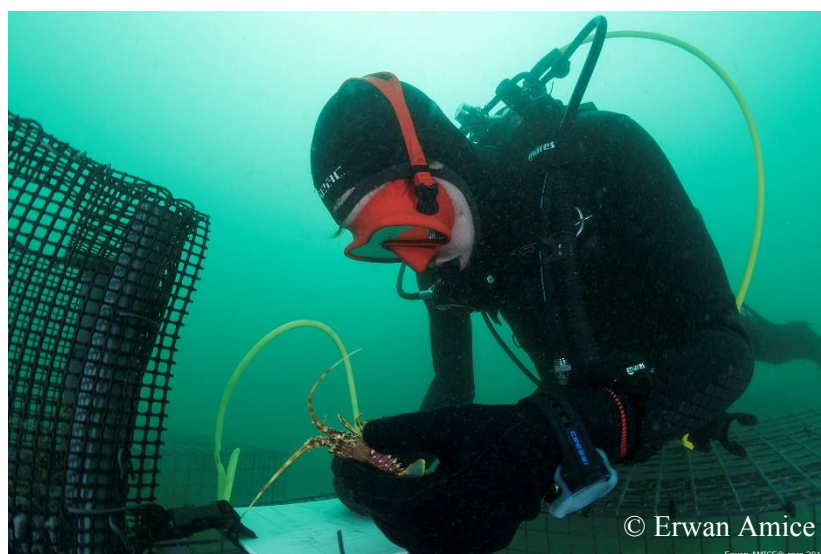
Conclusion

We have revisited the bioacoustics of *Palinurus elephas* and more generally that of spiny lobsters as a group. First, we have confirmed that broadband sounds, such as the antennal rasps, cannot be accurately characterized in tanks (except for the temporal features used in this study) because of sound reverberation and tank resonant frequencies. Indeed, the tank properties distort shapes of broadband sounds. In contrast, antennal rasps from spiny lobsters can be characterized accurately when recorded directly in their natural environment (*in situ*). We have provided 3 different types of sound features: temporal, intensity and spectral features. Secondly, we have shown for the first time that antennal rasps are among the loudest sounds produced among marine animals. We have also highlighted that their acoustic power is present in a low frequency (below 1 kHz); such low frequency content was missed in previous studies that were conducted in tanks. Using these new results, we could suggest a new biological hypothesis concerning their ecological roles, in particular for intra-specific communication, that has been overlooked in the bioacoustics literature. Finally, we clearly demonstrated that these sounds have suitable properties for *in situ* passive acoustic monitoring. This could contribute to additional *in situ* behavioral studies to better understand *P. elephas* movement patterns. This could also be developed as a tool for the management of *P. elephas* fisheries, especially to permit detection of areas with juveniles in order to protect them from destructive fishing practices (Goñi and Latrouite 2005).

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Chapitre 5 : Caractéristiques sonores de différentes tailles de langoustes dans le milieu marin : niveaux sources, propagation et distances de détection



Résumé

Nous avons mis en évidence dans le Chapitre 4 le fort potentiel acoustique de la langouste rouge pour des suivis *in situ* par acoustique passive. Avant d'arriver à un tel outil, il est nécessaire d'estimer les distances de détection entre des langoustes et un hydrophone, car elles peuvent donner des informations importantes sur leur distribution spatiale dans le milieu marin. Ces calculs utilisent des mesures de niveau source (*i.e.* une estimation du niveau acoustique à 1 m de l'animal), et des pertes de transmission (*i.e.* l'atténuation du son lorsqu'il se propage). A l'inverse des mammifères marins et des poissons, les études de propagation des sons produits par les crustacés *in situ* sont rares et ne se basent que sur des modèles théoriques simples (e.g. Radford et al. 2011, Butler et al. 2017). Cependant, ces modèles ne reflètent pas précisément la propagation des sons dans des environnements aussi complexes et hétérogènes que les milieux peu profonds où habitent la plupart des crustacés (Richardson et al. 1995). Dans ce contexte, nous avons utilisé une antenne d'hydrophones de 100 m de long dans le but de mesurer précisément la propagation des sons produits par *P. elephas* dans une baie côtière. Ce protocole, totalement novateur pour la bioacoustique des crustacés, nous a permis de calculer des niveaux sources et pertes de transmission des rasps d'antennes de langoustes de différentes tailles dans le but d'estimer leurs distances de détection.

Nous avons tout d'abord montré que les niveaux sources des rasps d'antennes sont extrêmement puissants. En effet, nos mesures placent la langouste rouge comme le deuxième crustacé le plus bruyant jamais reporté dans la littérature bioacoustique (après la crevette pistolet). De plus, les niveaux sources variaient significativement et positivement avec la taille des individus, avec une différence de 35 dB entre la plus petite (~ 20 g) et la plus grosse langouste (~ 2 kg). Ces variations d'intensité sonore sont connues dans la littérature bioacoustique des insectes qui possèdent des mécanismes de production de sons similaires aux

Partie 2 - Chapitre 5 : Propagation des sons produits par *P. elephas* dans le milieu marin

langoustes (Patek 2001, Sandborn and Phillips 1995). Ces différences pourraient s'expliquer par l'évolution ontogénétique des structures produisant les sons chez les langoustes.

A contrario, les modèles calculés de pertes de transmission n'étaient pas corrélés significativement avec la taille des langoustes. Ce résultat était attendu : la physique de la propagation des sons ne dépend pas de la source sonore, et est donc indépendante de la taille de l'animal. De plus, les modèles calculés étaient cohérents avec la littérature acoustique sur la propagation des sons en eau peu profonde (Rogers and Cox 1988, Bass and Clark 2003), mais différents des modèles théoriques largement utilisés dans la littérature bioacoustique. Nos résultats confirment donc la nécessité de mesurer (ou modéliser) précisément la propagation des sons produits par les crustacés dans de tels environnements avant toute généralisation.

Les mesures précises des niveaux sources et des pertes de transmission offrent la possibilité d'estimer les distances auxquelles il est possible de détecter un animal avec un hydrophone dans le milieu marin. Comme les niveaux sources dépendent de la taille des individus, les distances de détection varient également avec la taille. Dans les conditions de bruits ambiants enregistrés durant l'expérimentation, ces distances de détection variaient de quelques mètres pour les plus petites langoustes à plus de 400 m pour les plus grosses. Le bruit ambiant étant élevé à cause de la présence d'activité humaine à proximité du site d'étude, nous avons ensuite utilisé des valeurs théoriques de bruit ambiant naturel (Wenz 1962). De plus, les grosses langoustes étant détectées par le dernier hydrophone placé à 100 m, nous avons estimé leur propagation lointaine à l'aide de modèles théoriques (Richardson et al. 1995). Dans ces conditions, les rasps d'antennes produits *P. elephas* pourraient être détectés à l'échelle du kilomètre dans le milieu marin, avec les sons des plus grosses langoustes pouvant être détectés à plus de 3 km. Ces résultats seront précieux pour de futures études utilisant l'acoustique passive, car ils pourraient permettre de potentiellement détecter, localiser et même estimer des densités de langoustes sur de grandes échelles spatiales et temporelles.

Enfin, nous avons étudié l'évolution du spectre des antennal rasps en fonction de la distance des langoustes. Comme dans le Chapitre 4, les sons étaient tous (sauf pour les plus petits individus) dominés par les basses fréquences (< 1 kHz) proche de la source (< 10 m). Cependant, au-delà de 10 m, ce contenu basse fréquence était masqué par le bruit ambiant intense, laissant apparaître les trains d'impulsion comme des signaux haute fréquence (> 10 kHz). En supposant que les langoustes détectent uniquement les basses fréquences comme cela a été montré chez certains crustacés (Budellmann 1992, Popper et al. 2001, cf **Chapitre 3**), cela signifie qu'elles ne pourraient communiquer que sur de courtes distances (*i.e.* < 10 m) dans de tels environnements.

Article n°5:

Spiny lobster sounds can be detectable over kilometers underwater

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ABSTRACT

The detection ranges of broadband sounds produced by marine invertebrates are not known. To address this deficiency, a linear array of hydrophones was built in a shallow water area to experimentally investigate the propagation features of the sounds from various sizes of European spiny lobsters (*Palinurus elephas*), recorded between 0.5 and 100 m from the animals. The peak-to-peak source levels (SL, measured at one meter from the animals) varied significantly with body size, the largest spiny lobsters producing SL up to 167 dB re 1 μPa^2 . The sound propagation and its attenuation with the distance were quantified using the array. This permitted estimation of the detection ranges of spiny lobster sounds. Under the high ambient noise conditions recorded in this study, the sounds propagated between 5 and 410 m for the smallest and largest spiny lobsters, respectively. Considering lower ambient noise levels and different realistic propagation conditions, spiny lobster sounds can be detectable up to several kilometres away from the animals, with sounds from the largest individuals propagating over 3 km. Our results demonstrate that sounds produced by *P. elephas* can be utilized in passive acoustic programs to monitor and survey this vulnerable species at kilometre scale in coastal waters.

INTRODUCTION

Passive acoustic monitoring (PAM) of marine species has recently gained attention by biologists and is now used worldwide. This is due to the increased knowledge of animal sound repertoires, and the behavioral contexts in which they are produced (Tyack and Clark 2000, Lobel et al. 2010, Tricas and Boyle 2014). In addition, the density of seawater enables sounds to propagate over greater distances compared to air (Urlick 1983). Estimating the detection ranges between a particular sound-producing species and a receiver can give crucial information about its spatial distribution in an ecosystem. These calculations rely on the measurements of the source level (SL, *i.e.* the sound pressure level recorded at 1 m from the source) and the transmission loss (TL, *i.e.* the attenuation of the sound as it propagates away from the source) of animal sounds underwater. For example, marine mammal sounds can be detected kilometres away in shallow and deep oceans with hydrophones (Stafford et al. 1998, Zimmer et al. 2008, Bonnel et al. 2014). Fish also produce sounds in shallow waters that can be detectable from few meters (Mann and Lobel 1997, Alves et al. 2016) to hundreds of meters away (Sprague and Luczkovich 2004, Locascio and Mann 2011). However, data available on sound propagation and detection ranges for crustaceans are scarce, though crustaceans are known to emit a large diversity of sounds (Schmitz 2002, Jézéquel et al. 2018, Jézéquel et al. 2020).

Marine arthropods produce sounds that are mostly characterized by broadband pulses, *i.e.* short transient sounds with a large bandwidth (Meyer-Rochow and Penrose 1976, Au and Banks 1998, Jézéquel et al. 2019). Estimating their sound propagation may be challenging as they inhabit shallow coastal waters (at depths below tens of meters). This implies complex sound fields due to physical constraints such as the presence of boundaries created by the water surface and the seabed (Rogers and Cox 1988, Bass and Clark 2003), and it is thus difficult to accurately model sound propagation (Richardson et al. 1995). Until now, detection ranges of crustacean sounds have relied on crude estimations of SLs performed using distant

measurements that are then artificially back-propagated to 1 m by using theoretical propagation models (Radford et al. 2011, Butler et al. 2017). In addition, some studies have been performed in tanks. Tank experiments are very convenient since distances between receivers and animals can be precisely measured (Coquereau et al. 2016). However, tank acoustics are complicated. The relatively small volumes and close boundaries of tanks highly affect sound propagation as well as SL estimates (Jézéquel et al. 2018, 2020) ; these tank effects have been largely ignored by most previous studies (as a counter example, see Jézéquel et al. 2019 for an experimental illustration on the differences of crustacean sounds recorded in tanks and *in situ*). Thus, there is a need to combine theory with empirical measurements of site-specific sound propagation to obtain reliable SL and TL predictions for estimating detection ranges of crustacean sounds. For this purpose, arrays of hydrophones are useful because they can accurately estimate SL and TL in shallow waters (Madsen and Wahlberg 2007, Locascio and Mann 2011).

Estimating SL requires an accurate knowledge of the distances between sound producers and receivers. This problem is likely easier for many benthic crustaceans than for marine mammals and fish, since they have a relatively low mobility. Spiny lobsters are particularly good models for performing such studies because they produce specific sounds termed “antennal rasps”. While these antennal rasps are characterized by trains of broadband pulses with sound intensity spread over a wide bandwidth, their spectrums are dominated by low frequencies (i.e. below 1 kHz; Patek et al. 2009, Jézéquel et al. 2019). In addition, it is possible to induce sound production by handling the animals underwater (which aims to imitate a predator attacking; Meyer-Rochow and Penrose 1976, Patek et al. 2009, Jézéquel et al. 2019). Hence, these animals are practical for accurately measuring SL and propagation of produced sounds over different distances, while precisely controlling their positions. Interestingly, sounds produced by spiny lobsters have similarities with insects (Patek 2001). Several studies in terrestrial arthropods have shown that the intensity of their sounds depends on body size

Partie 2 - Chapitre 5 : Propagation des sons produits par *P. elephas* dans le milieu marin (Sanborn and Phillips 1995, Bennet-Clark 1998). If such a relation also exists in spiny lobsters, this implies that larger individuals likely produce higher amplitude sounds compared to smaller animals. As a result, these large individuals should be detectable over longer distances. In addition, a recent study recorded antennal rasps from the European spiny lobster (*Palinurus elephas*) underwater and reported high peak-to-peak sound pressure levels (SPL) up to 170 dB re 1 μPa^2 at 20 cm from the animals (Jézéquel et al. 2019). Such elevated SPLs imply these sounds could be detectable during *in situ* PAM studies, which is needed to better manage this highly commercially valuable and vulnerable species that has become scarce in European coastal waters due to overfishing (Goñi and Latrouite 2005, Goñi 2014). However, it is now crucial to understand the variability of their sounds (*i.e.* with animal size), how they propagate and at what distances they can be detectable underwater with hydrophones before PAM can be used operationally to monitor spiny lobsters.

In this context, the aim of this study was to provide new insights on the propagation features of broadband sounds produced by a marine crustacean, the European spiny lobster (*P. elephas*), in a shallow coastal water area. First, we measured the SPLs of individuals from various sizes (from 2.6 to 13.5 cm of carapace length) using a linear array of 8 hydrophones placed between 0.5 and 100 m from the animals. Using this set-up, the SLs, in terms of SPLs, were obtained at 1 m from the animals. Secondly, we estimated the associated TLs using a simple model $a \times \log_{10}(r)$, with r being the source-receiver distance and a being the model parameter to be calculated, and we compared the estimated TLs with theoretical models. Based on these results, the detection ranges (and their variability with animal size) were estimated using different conditions of ambient noise levels (ANL) and TL models. Lastly, we examined changes in the spectral contents of the antennal rasps with increasing distance from the spiny lobsters, and discussed their potential ecological implications.

Materials and Methods

Animal collection, characteristics and care

For the experiments, we used a total of 24 *P. elephas* individuals of a wide range of sizes. Only inter-moult individuals with full sets of intact appendages were selected for this study.

We carefully collected 17 juveniles by hand while scuba diving in the Bay of Perros Guirec (48°50'2.044" N, 3°26'28.312" W) at depths between 10 and 25 ms during two diving sessions on May 28th, 2019. Juvenile individuals (3 males and 14 females) had carapace lengths (CLs) between 2.6 and 8 cm, as measured from the anterior tip of the rostrum to the medial point of the posterior carapace margin. Large adult individuals were bought from local fishermen several days after they were captured in the Iroise Sea on May 21st, 2019. These 7 large spiny lobsters (2 males and 5 females) had CLs between 9.5 and 13.5 cm.

After capture, all individuals were immediately transferred to an isolated, quiet room in the facilities of the Institut Universitaire Européen de la Mer (IUEM) in Plouzané (France). They were placed in holding tanks of different dimensions according to their size: three plastic-sided rectangular tanks (0.60 m × 0.50 m × 0.35 m, length × width × effective height; seawater volume = 0.1 m³) and two plastic-sided square tanks (1.0 m × 1.0 m × 0.6 m, 0.6 m³). There were 4 to 8 individuals per holding tank. Before they were placed in these tanks, all individuals were tagged using alternating small white and black rubber bands placed on the base of their second antennae. Each tagged individual was described (size, sex) and given an identification number.

Holding tanks were continuously supplied with the same sand-filtered seawater pumped from the Bay of Brest. During the holding period, temperature was 14.6 ± 0.6 °C and salinity was 34.7 ± 0.1 . All animals were fed with fresh pieces of fish (mackerel) *ad libitum*. They were kept under a 12:12 photoperiod; daylight conditions (from 08:00 am to 08:00 pm) were simulated with fluorescent light tubes placed above the holding tanks. Several sections of rigid,

PVC pipes associated with large rocks were provided as shelters in each tank. Animals were acclimatized at least 15 days in these holding conditions before they were used in the at-sea recording experiment.

Sound recordings and video

A linear array of 8 hydrophones was used to record sounds produced by the spiny lobsters (see Table 8 for characteristics of the recording devices). The 8 hydrophones were spaced relative to the handled spiny lobsters at 0.5, 1, 5, 10, 15, 20, 50 and 100 m. The 2 closest hydrophones (0.5 and 1 m) were set with a gain of 0 dB which permitted us to characterize the powerful antennal rasps without clipping the recorded sounds (*i.e.* sound saturation).

Table 8: Characteristics of the recording devices used for the linear array of hydrophones (placed between 0.5 and 100 m from the spiny lobsters) during in situ recordings. Fs: frequency sampling.

Hydrophone		Recorder				
Model	Flat frequency response (kHz)	Sensitivity (dB re 1 V μPa^{-1})	Model	Gain (dB)	Fs (kHz)	Distance from the spiny lobsters (m)
HTI-99-HF	0.002 - 125	-174.9		0	156	0.5
		-174.7	EA-SDA14	0	156	1
		-174.9	(RTSys, France)	15	156	5
		-175		15	156	10
		-174.8	EA-SDA14	15	156	15
		-174.7	(RTSys, France)	15	156	20
HTI-96-MIN	0.002 - 30	-163.8	Wildlife Acoustics Song Meter (Model SM2)	24	96	50
HTI-92-WB	0.002 - 50	-155.5	EA-SDA14 (RTSys, France)	15	156	100

Video recordings were made during *in situ* recordings using two GoPro HERO3 cameras at a recording rate of 29.97 frames per second. The videos allowed confirmation of sound production by each spiny lobster tested, and also provided the time at which the handled individuals were placed at the source point during the sound recordings. They also validated the identification of each tested individual by checking the rubber bands on the spiny lobsters' second antennae.

Location and characteristics of the experimental site

The experimental site where sound recordings were performed was located in the Bay of Saint Anne de Portzic (48°21'32.951" N, 4°32'59.024" W) in the Bay of Brest, just beneath the facilities of the IUEM where spiny lobsters were held. It was located about 100 m outside a marina hosting 120 recreational boats. It is a shallow water area with depths varying between 15 m during high tide and 9 m during low tide. The bottom is flat and composed of homogenous, fine sand with empty shells.

Experimental set up

The day prior the recording experiment, while scuba diving, all spiny lobsters were transferred into three galvanized steel cages (1.0 m × 1.0 m × 0.5 m, 0.5 m³) placed side by side linearly on the bottom near a rocky dyke. Sections of rigid PVC pipes were provided as shelters. Spiny lobsters were acclimatized for 24 hours in these conditions to recover from transport and handling.

The next day (June 14th, 2019), while scuba diving, the linear array of hydrophones was built in front of the center holding cage. First, a rope was laid on the substrate, which was previously marked at each distance where the different hydrophones should be placed. Then, hydrophones were attached 0.5 m above the bottom to metal rods anchored with concrete tubes at each mark placed on the rope. Cables were anchored to the bottom with lead weights and recorders were laid on the bottom. Because the Wildlife recorder had a positive buoyancy, it

was anchored to the bottom using a lead weight. Thus, its hydrophone (placed at 50 m from the spiny lobsters) was located at 1 m above the bottom. The two cameras (same model GoPro as mentioned above) were placed on the top of the 2 outside cages, in front of the center cage. Then, the boat transporting scuba divers was anchored 200 m away from the cages, and its motor was shut down. Sound recordings were performed during low tide to avoid tidal currents. Before the recording experiment started, the ambient noise was recorded for 10 minutes without scuba divers underwater. Next, each spiny lobster was gently picked up, handled one by one, and positioned at the source point. The source point, defined as the point where spiny lobsters were recorded, was located at the beginning of the rope, at 0.5 m from the first hydrophone. Each individual was maintained at the same distance above the bottom (0.5 m) as the hydrophones during recordings, and the spiny lobsters were held so that they faced the linear array of hydrophones. Thus, the body of the animals was on the same axis as the linear array of hydrophones. We chose to handle spiny lobsters to elicit their sound production, as this method is commonly described in the bioacoustic literature on spiny lobsters (Meyer-Rochow and Penrose 1976, Patek et al. 2009, Jézéquel et al. 2019). Each sound recording for the different spiny lobsters lasted between 20 and 30 s. During each sound recording, the two scuba divers stopped their breath to avoid the emission of intrusive noise related to air bubbles. In total, the recording experiment lasted 60 min. In the end of the recordings, five sharp raps were made on the cage walls which permitted us to synchronize both hydrophones and GoPros.

Sound analysis

Sound features of antennal rasps

Synchronized recordings of sounds (in .wav format) and videos were first analyzed to confirm sound production by each tagged spiny lobster. Then, each antennal rasp was extracted manually using the Audacity software (version 2.1.1, Audacity Team Audacity®). Antennal rasps were defined as pulse trains composed of at least several pulses separated by less than 20

ms from each other. Hence, any isolated pulses present in the recordings were not analyzed here. We performed sound analysis on a total of 10 antennal rasps per spiny lobster and per distance (80 sounds analyzed per individual in total). The same sounds were analyzed at the 8 different distances for each spiny lobster. All sequences were then processed using custom MATLAB scripts (version 9.1; The MathWorks).

We calculated the intensity features of the antennal rasps based on their sound pressure levels (SPL, in dB re $1 \mu\text{Pa}^2$) both in peak-to-peak (for biological interpretation) and root-mean-square (for detection range estimations using the passive sonar equation).

As these sounds are pulse trains characterized by short and transient pulses, we first chose to calculate the peak-to-peak SPL (SPL_{pp}) which is the most representative and practical intensity feature for these types of biological sounds (Jézéquel et al. 2019, Madsen 2005b). As we recorded antennal rasps at several distances from the spiny lobsters, the SPL calculated at 1 m was referred to as the source level (SL_{pp}). When pulse trains were affected by low frequencies related to ambient noise (below 50 Hz), especially at long distances (50 and 100 m), we measured the SPL_{pp} based on the pulse with the highest and lowest amplitude of the train to avoid overestimating their values. When pulse trains could not be isolated from the ambient noise, we did not calculate their SPL_{pp} . We then averaged the SPL_{pp} calculated per distance and per individual for further analysis. The SPL_{pp} and SL_{pp} were used for biological sound characterization. Because these values varied according to the body size of the spiny lobsters (see Results), we chose to regroup the averaged values per group of size-matched individuals for a better overall description. Four different groups of body sizes were defined and termed as follow: large ($8.0 < \text{CL} < 13.5$ cm, $N = 9$), intermediate ($6.4 < \text{CL} < 7.3$ cm, $N = 7$), small ($4.2 < \text{CL} < 4.8$ cm, $N = 3$) and very small ($2.6 < \text{CL} < 3.1$ cm, $N = 5$).

We also calculated the SPL and SL as root-mean-square (SPL_{rms} and SL_{rms} , respectively) by integrating the power spectral density (PSD, in dB re $1 \mu Pa^2 \cdot Hz^{-1}$) of the antennal rasps between 10 and 78 kHz (bandwidth where intensity from the antennal rasps remained above 10 m from the spiny lobsters, see Results). The SPL_{rms} at 50 m was calculated over the 10-48 kHz frequency band, because the system specification did not allow measurements of frequencies above 48 kHz (see Table 2). Because antennal rasps are characterized by pulse trains, the SPL_{rms} and SL_{rms} values averaged on the entire length of the antennal rasps would be underestimated. We instead chose to calculate them on each pulse inside the pulse trains over a 1 ms window length (Fast-Fourier Transform size: 156 points) centered on the pulse. Then, each value was averaged over all pulses present in an antennal rasp to obtain its mean SPL_{rms} and SL_{rms} . As for SPL_{pp} and SL_{pp} , the SPL_{rms} and SL_{rms} values were averaged on 10 antennal rasps per individual. The SPL_{rms} and SL_{rms} values were used to estimate transmission loss (TL), as well as to compute the passive sonar equation to estimate detection ranges (Ainslie 2010).

We also calculated the dominant frequency (in kHz) of each antennal rasp, represented as the frequency where the PSD was maximal. At large distances, some sounds were lost in the ambient noise while looking at the time domain signals, their SPL_{pp} and SPL_{rms} were thus not computed. However, they were still visible in the frequency domain; in this case, their dominant frequency was estimated.

Ambient noise characterization

Recordings of ambient noise (10 min each) from the 8 hydrophones were first visualized to ensure the absence of antennal rasps. Because anthropogenic noise affected the ambient noise recordings during the experiment, sound sequences were both cut into 20 sequences of 30 seconds each, and we randomly selected 3 of 20 sequences from each recording. The sequences where anthropogenic noise (mainly shipping noise) was dominant were not taken into account in the analysis. We calculated the SPL_{rms} of all selected 30 s long sequences. This SPL_{rms} was

calculated over the same frequency band as the antennal rasp SPL_{rms} and SL_{rms} (10-78 kHz), except for data from the Wildlife recorder, for which SPL_{rms} was calculated over the 10-48 kHz frequency band. This provided a mean value for the ambient noise at each hydrophone placement, and was referred to the ambient noise level (ANL).

Evaluation of transmission losses

The datasets of the averaged SPL_{rms} for each individual and for each distance were fitted with nonlinear least-squares regressions using custom-made scripts in MATLAB. We used the following equation (Ainslie 2010):

$$SPL_{rms} = SL_{rms} - TL \quad \text{Eq.1}$$

where TL is the transmission loss (in dB). TL represents the loss of intensity due to the geometrical spreading of sounds in a physical medium (Urick 1983), and was calculated as the slope of the logarithmic regression between SPL_{rms} and the distance from the spiny lobsters, which was expressed as:

$$TL = a \times \log_{10}(r) \quad \text{Eq. 2}$$

where r is the distance between the spiny lobsters and the hydrophones (in meters), and a is the geometrical TL term.

We obtained 19 different TL models using this method on the dataset generated by each animal (i.e. known SPL_{rms} , SL_{rms} and r). The measurements from the 5 very small individuals were not included in this analysis because we did not have enough measurement points as they were only detectable between up to 5 and 10 m (see Results). Moreover, a global TL coefficient was also estimated using a global dataset obtained by merging the sounds from the 19 spiny lobsters. We compared this global TL with other theoretical models of TL commonly used in the bioacoustic literature (Bass and Clark 2003). In theory, the spherical spreading loss ($TL = 20 \times \log_{10}(r)$) prevails near the source where sound propagates uniformly in all directions.

The cylindrical spreading loss ($TL = 10 \times \log_{10}(r)$), prevails in shallow waters where sound cannot propagate as a spherical wave in all directions but only as a cylindrical wave bounded by the sea floor and the sea surface. We also used a combined TL, termed the ‘practical’ spreading loss ($TL = 15 \times \log_{10}(r)$), which occurs between the prediction of the two other spreading models described above (Coates 1989).

Estimations of detection ranges

For the purpose of this study, we assumed that signal detection by hydrophones was primarily limited by the TL (previously calculated), the ANL and the absorption (α) of the high frequencies considering the detection ranges (i.e. kilometre scale).

Using the previous results, we estimated the detection ranges of the antennal rasps by resolving the passive sonar equation (in the frequency domain) for all 24 spiny lobsters (Ainslie 2010):

$$SL_{\text{rms}} - TL - \alpha - \text{ANL} = \text{SNR} \quad \text{Eq. 3}$$

Where:

- SL_{rms} is the source level in dB re $1 \mu\text{Pa}^2$ (in root-mean-square; averaged on 10 measurements per spiny lobster), calculated in the 10-78 kHz frequency band (Ainslie 2010).
- TL is the global coefficient of TL previously calculated for the 19 largest spiny lobsters. We also used the models of cylindrical and practical TL detailed above.
- α is the coefficient of attenuation, depending on the frequency of the sound. Here, it was used at the dominant frequency that was commonly found above 10 m in the recorded antennal rasps, which was estimated at 30 kHz (see Results). Thus, the coefficient of absorption was calculated to be 7 dB per km (Fisher and Simmons 1977).

- ANL is the ambient noise level in dB re $1 \mu\text{Pa}^2$ (in root-mean-square) calculated over the same band of frequencies than the SL_{rms} (10-78 kHz). We used two different values of ANL. First, we reported the mean ANL recorded by our hydrophones during the study. Second, we used one theoretical (but still realistic) value of ANL based on Wenz curves and calculated with a wind speed of 5 knots in the same frequency band than the ANL *in situ* (Wenz 1962).
- SNR is the signal-to-noise ratio which corresponds to the minimum threshold needed for the hydrophones to detect the sound above the ANL. We used two different SNRs of 5 and 10 dB widely accepted for sonar systems (Ainslie 2010).

Because our sound recordings were performed in shallow waters (< 10 m), we considered the water column as non-stratified. Thus, the effects of sound speed were assumed to be independent of depth (Urick 1983), and were not taken into account in this equation. Also note that in theory, the absorption coefficient α should be embedded into our estimated TL model. However, the impact of α is relatively small over our array range, with a loss smaller than 1 dB, which is negligible with respect to the geometrical TL. On the other hand, at larger ranges, the impact of α becomes important. We thus decided to add α in Eq. (3); this ensures that detection ranges are not over estimated.

Statistical analysis

We examined the correlations between SL_{pp} and TL with body size using Pearson tests ($\alpha = 0.05$). Analysis were performed using R version 3.5.1 (R Core Team).

Ethical statement

Experiments with European spiny lobsters are not subject to restriction for animal scientific research according to the French legislation and the European Community Council Directive of September 2010 (2010/63/UE). However, we followed the ARRIVE guidelines (Kilkenny et al. 2010) to ensure that all experiments were performed under good conditions. Animals were handled with care during the study and their health status were checked daily by the authors. No specimens were harmed during this study and there was no mortality. At the end of the study, 7 adults were kept in the laboratory for other experiments. All the other animals were released back into the environment where they were collected.

RESULTS

During the experiment, the wind state ranged between 0 (calm) and 2 (light breeze) on the Beaufort scale, corresponding to speeds between 1 and 6 knots. Seawater temperature was 15.3 °C and salinity was 35. The water depth was 9 m. Anthropogenic noise from a near marina contributed to the low frequencies (below 1 kHz) in the recorded ambient noise, and was mainly produced by ship motor noise and the chains of the boats' buoys rubbing against the bottom. However, the ANL in the frequency band from 10-78 kHz was quieter compared to the ANL calculated over the entire (0.001-78 kHz) frequency band (over 20 dB difference), and varied slightly across the different hydrophones with a mean of 90 ± 4 dB re $1 \mu\text{Pa}^2$. The sources of ambient noise in the frequency band 10-78 kHz were attributed to isolated broadband pulses from unknown sources.

We extracted and analyzed manually a total of 1560 antennal rasps from the sound recordings of spiny lobsters ($N = 24$) with the linear array of 8 hydrophones placed between 0.5 and 100 m. This total number of analyzed antennal rasps corresponds to the 1920 antennal rasps that were recorded (10 per animal and per recording distance), minus 360 antennal rasps that were not analyzed because the signal-to-noise ratio was too low (i.e. the antennal rasps were buried in the ambient noise). Indeed, antennal rasps from intermediate ($N = 7$), small ($N = 3$) and very small ($N = 5$) individuals were not recorded beyond 50, 20 and 5 m, respectively (see Figure 18). In marked contrast, all sounds from large spiny lobsters ($N = 9$) were recorded on all the hydrophones, with distance up to 100 m.

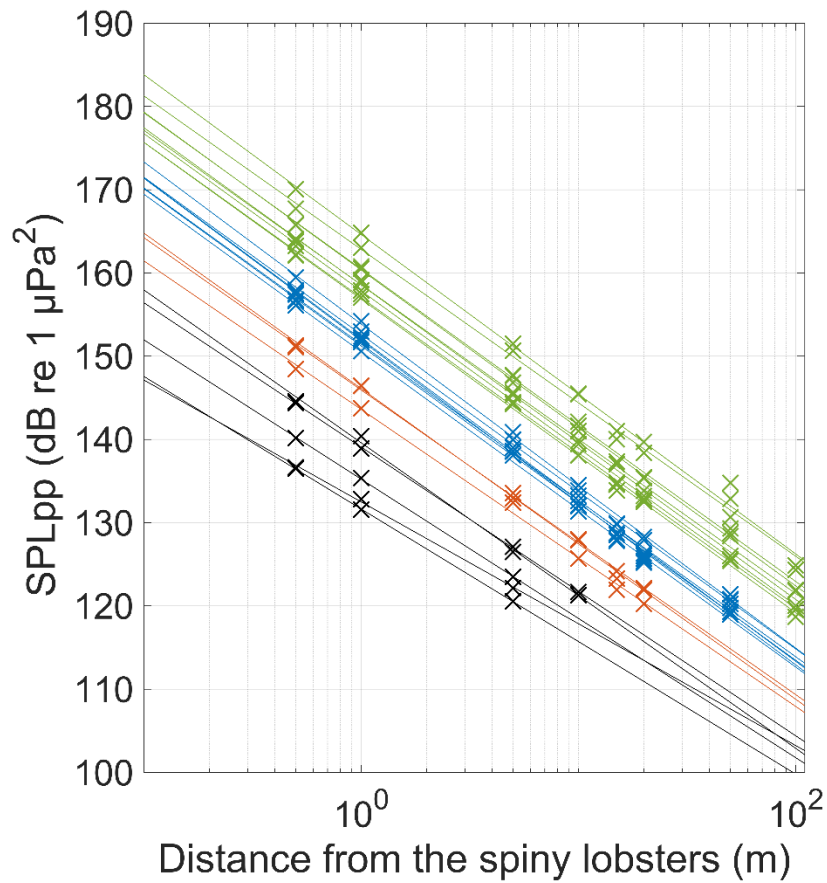


Figure 18: Evolution of sound pressure levels in peak-to-peak (SPL_{pp} ; crosses) recorded at different distances from the spiny lobsters (between 0.5 and 100 m) and their calculated fit curves using logarithmic models (continuous lines). Each point represents the mean SPL_{pp} averaged on 10 sounds. The x-axis is in logarithmic scale. Each value calculated at 1 m corresponds to the source level (SL_{pp}) of the spiny lobsters. The colors are related to the body size of the spiny lobsters (green: large, blue: intermediate, orange: small, black = very small).

The SL_{pp} varied significantly and positively according to the body size of spiny lobsters (Pearson: $r = 0.910$, $t = -10.316$, $N = 24$, $df = 22$, $p < 0.001$). Indeed, we calculated a maximum difference of 35 dB between the smallest ($SL_{pp} = 132$ dB re $1 \mu Pa^2$; $CL = 2.6$ cm) and the largest (167 dB re $1 \mu Pa^2$; $CL = 13.5$ cm) spiny lobsters (Figure 18). The maximum calculated SPL_{pp} was 172 dB re $1 \mu Pa^2$ and was produced by the largest individual ($CL = 13.5$ cm) at 0.5 m.

The TL models estimated as $a \times \log_{10}(r)$ from the dataset of SPL_{rms} vs. distance did not significantly vary with body size (Pearson: $r = -0.175$, $t = -0.733$, $N = 19$, $df = 17$, $p = 0.474$), as expected. The estimated TL parameter a ranged between 16.1 and 19.5 (Figure 19). By fitting the results amongst all individuals (except the very small ones), the global TL parameter a was 17.6 which is between the theoretical models of practical ($a = 15$) and spherical ($a = 20$) TLs (Figure 19).

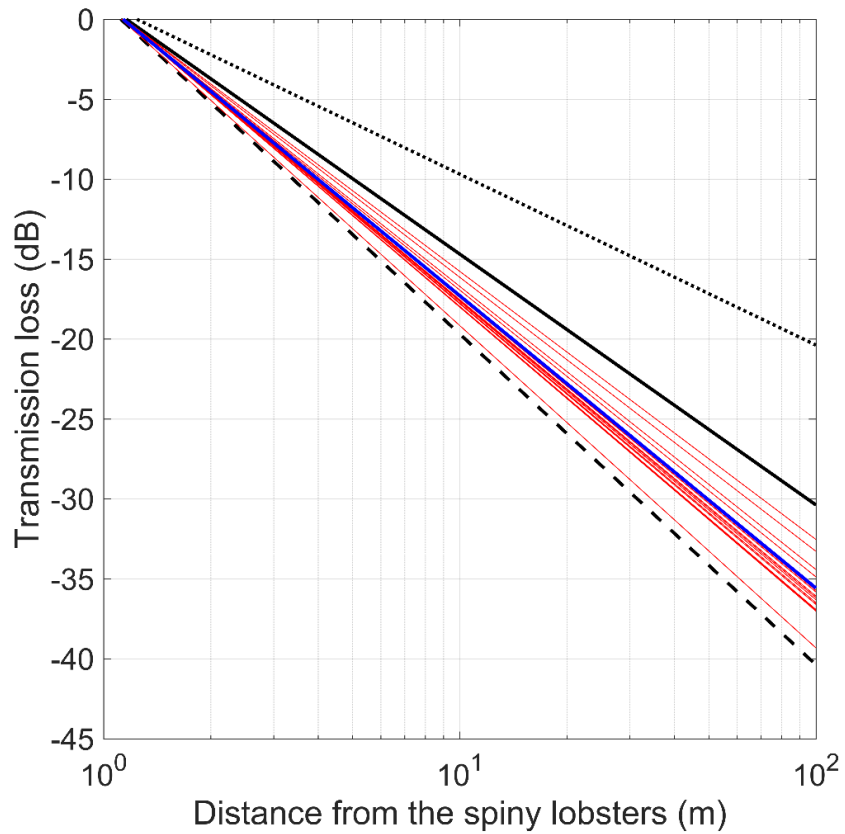


Figure 19: Transmission losses (TL) estimated for 19 spiny lobsters (red lines) based on the dataset SPL_{rms} vs. distance, and the global fitted TL (blue line, $a = 17.6$). Black lines represent theoretical models of TL: dotted is cylindrical ($a = 10$), continuous is practical ($a = 15$), dashed is spherical ($a = 20$).

Detection ranges of the antennal rasps produced by all spiny lobsters were estimated per group of body size by considering the calculated SL_{rms} and global TL ($a = 17.6$). Their values are summarized in Table 9. Under the ANL conditions encountered during the experiment, we estimated that large individuals can be recorded up to 250 m, with the largest individual (CL = 13.5 cm) being detectable up to 410 m (Table 9). Interestingly, the estimated detection ranges for smaller individuals are less than 100 m, and are thus covered by the range of our array. Thus, these estimated values are consistent with the values observed on the *in situ* recordings. Indeed, intermediate, small and very small spiny lobsters could not be recorded at 100, 50 and 5 m (respectively), which matches our estimations of detection ranges (Table 9). In a theoretical low (but still realistic) ANL, large individuals can be detectable at 750 m, with the largest individuals (CL = 13.5 cm) being recorded up to 1080 m. By considering a practical loss model for TL (the most realistic), large spiny lobsters could be detectable at the kilometre scale under the theoretical ANL, with largest individuals being detected up to 1740 m (Table 9). Using an attenuated cylindrical loss model for TL (the least conservative), all spiny lobsters (except the very small ones) may be detectable at the kilometre scale under the theoretical low ANL, with largest spiny lobsters being detected up to 3610 m (Table 2).

Table 9: Estimations of detection ranges (in m) of antennal rasps produced by spiny lobsters underwater. The averaged values are reported per group of size-matched spiny lobsters (see Material and Methods for details). The different transmission losses (TL) used correspond to the global TL ($a = 17.6$) calculated between all spiny lobsters (except the very small ones), and the theoretical models of cylindrical TL ($a = 10$) and practical ($a = 15$) TLs. Min is signal-to-noise ratio (SNR) = 5 dB and max is SNR = 10 dB.

	TL	Wenz 5 knots		This study	
		Min ANL = 81	Max ANL = 86	Min ANL = 93	Max ANL = 98
Large (N = 9)	17.6	750	500	250	150
	15	1330	940	510	300
	10	3040	2450	1690	1180
Intermediate (N = 7)	17.6	390	230	100	60
	15	850	550	270	140
	10	2250	1710	1030	620
Small (N = 3)	17.6	210	120	50	27
	15	420	240	100	50
	10	1510	1030	500	240
Very small (N = 5)	17.6	70	40	15	8
	15	130	70	25	10
	10	620	330	100	40

Overall, a clear pattern occurred in changes of the spectral content of recorded antennal rasps with increasing distance. The spectral content was dominated by low frequencies (< 1 kHz) close to the spiny lobsters (< 10 m) whereas only high frequencies (> 10 kHz) remained far from the animals (> 10 m; Figures 20 and 21). However, antennal rasps produced by very small spiny lobsters did not present any low frequency content (< 1 kHz) even at 0.5 m (range 1.3 to 31.3 kHz). The low frequency content was probably masked by the ambient noise due to their low intensity features. Above 10 m, all recorded antennal rasps had dominant frequencies only between 10 and 60 kHz (Figures 20 and 21).

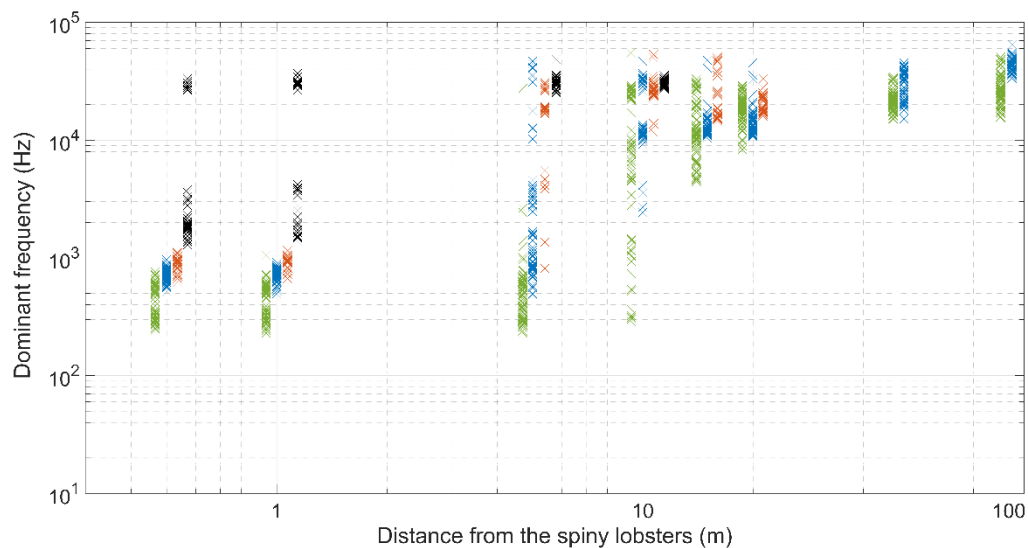


Figure 20: Dominant frequencies calculated on the recorded antennal rasps as a function of the animal-hydrophone distance. Each point represents the value from one antennal rasp. The colors are related to the body size of the spiny lobsters (green: large, blue: intermediate, orange: small, black: very small). Both x- and y-axis are in logarithmic scale.

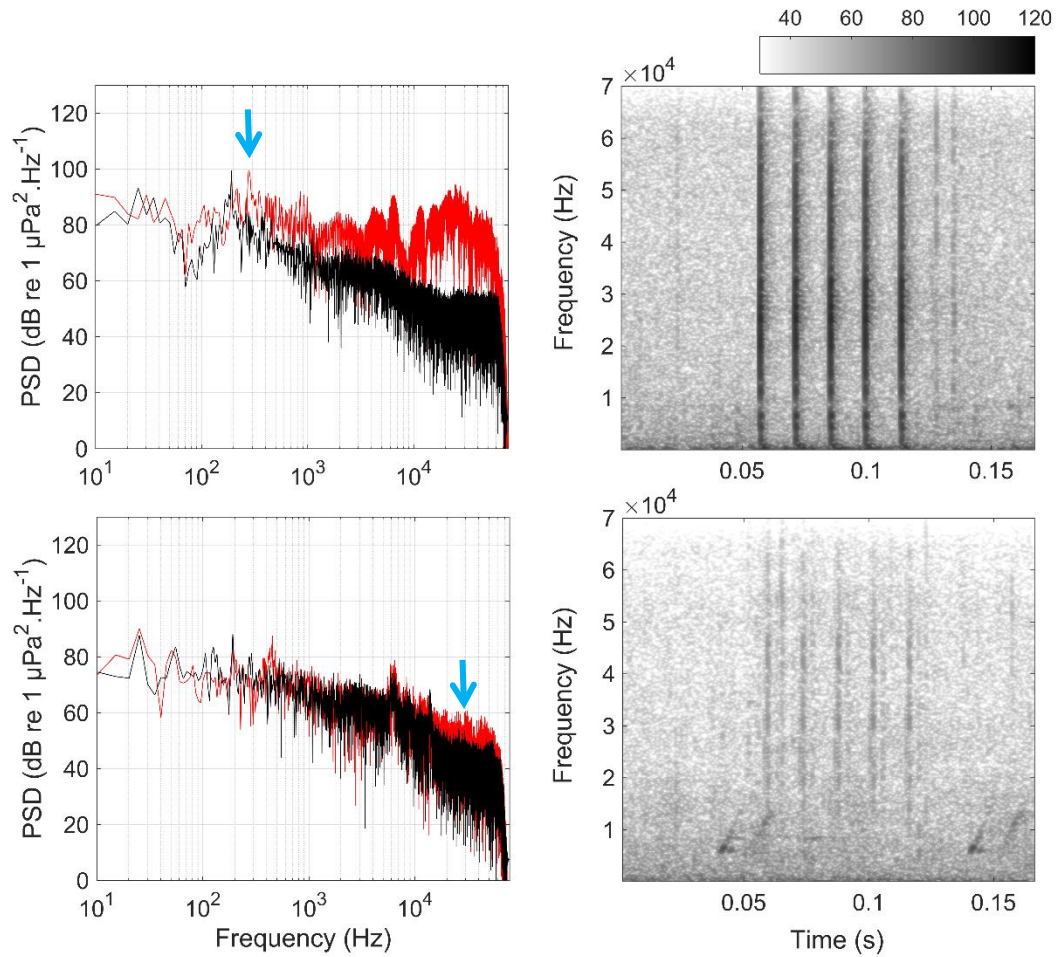


Figure 21: Power spectral densities (PSD, left) and spectrograms (right) of an antennal rasp produced by a large spiny lobster (carapace length = 13 cm) at 1 m (top) and 100 m (bottom). The black lines are the ambient noise recorded at the same distances. The blue arrows show the dominant frequencies of the recorded antennal rasp, calculated at 0.3 kHz at 1 m while it was calculated at 30 kHz at 100m. The color scale bar is in dB re 1 $\mu\text{Pa}^2 \cdot \text{Hz}^{-1}$.

Discussion

Passive acoustic monitoring

In the bioacoustic literature, only one study used passive acoustics to monitor spiny lobsters (*Panulirus japonicus*) underwater (Kikuchi et al. 2015). The authors found an increase in the production of antennal rasps on the nights of large tidal changes, which complement the ecological knowledge on the nocturnal behaviour of spiny lobsters (Herrnkind 1980). However, the propagation features of the recorded antennal rasps were not assessed, and thus the detection ranges at which spiny lobsters produced sounds were not quantified, which drastically limits the impacts of the corresponding study. To fill this gap, the present paper is the first to experimentally examine the propagation features of the antennal rasps produced by the European spiny lobster (*P. elephas*) in shallow waters using a linear array of hydrophones with a range of 100 m.

Although SL_{pp} values have been reported for several marine species of mammals and fish, this is the first time that SL_{pp} values for a marine crustacean are reported from direct *in situ* recordings. Spiny lobsters produce loud antennal rasps, with SL_{pp} up to 167 dB re $1 \mu Pa^2$ for the largest individuals (CL = 13.5 cm; Figure 19). Overall, our results confirm the range of values obtained by a recent study with the same species where SPL_{pp} (calculated only at 20 cm from the source) were calculated above 170 dB re $1 \mu Pa^2$ (Jézéquel et al. 2019). The antennal rasps produced by spiny lobsters have exceptionally elevated SL_{pp} among crustaceans. Indeed, only snapping shrimps have been reported to produce higher SL_{pp} , estimated in tanks up to 215 dB re $1 \mu Pa^2$ (Au and Bank 1998, Schmitz 2002). We also found that SL_{pp} of spiny lobsters vary significantly and positively with their body sizes, with larger individuals producing higher amplitude sounds compared to smaller individuals. Indeed, the smallest individuals (CL = 2.6 cm) had SL_{pp} calculated at 132 dB re $1 \mu Pa^2$, which was 35 dB less compared to the largest

individuals (CL = 13.5 cm; Figure 19). These variations in sound intensity with body size have already been described in insects whose sound production mechanisms are similar to spiny lobsters (Sanborn and Phillips 1995, Bennet-Clark 1998, Patek 2001). To our knowledge, no studies have examined ontogenetic variations of sound intensity in crustaceans. Further studies should relate these variations with the development of sound-producing structures in spiny lobsters.

The use of linear arrays of hydrophones is known to be useful to estimate SL and TL for marine mammals and fish (Madsen and Wahlberg 2007, Locascio and Mann 2011). In marked contrast to these highly mobile animals, spiny lobsters produce sounds while handling, which enables precise control of their distances from the recording hydrophones. This allowed us to perform accurate measurements of the SPL_{pp} and SPL_{rms} over distance from the spiny lobsters (between 0.5 and 100 m; Figure 19). While we found that SL_{pp} (and SL_{rms}) varied with body size, we did not find any significant relationship between the different coefficients of TL and the body sizes of the spiny lobsters. This result was to be expected: the physics of sound propagation does not depend on the sound source, and it is thus independent from the animal body size. The estimated TL [$17.6 \times \log_{10}(r)$] is consistent with sound propagation in shallow water (Rogers and Coxx 1988, Richardson et al. 1995, Bass and Clark 2003), but different from simplistic models (e.g. cylindrical losses) that are often used in bioacoustics. This clearly demonstrates the importance of correctly assessing sound propagation in shallow waters to study crustaceans' sounds. Last but not least, we only recorded sounds produced by spiny lobsters while facing the linear array of hydrophones (i.e. on the same axis). Thus, we did not quantify the potential directivity of their sound source. The use of three-dimension arrays will be useful in further studies to better describe the directivity and 3D propagation of antennal rasps in shallow waters (Locascio and Mann 2011).

An important application of measuring accurately SL and TL is the estimation of detection ranges at which animals can be detectable underwater with hydrophones. We found that the SLs of spiny lobsters varied with body size, and thus their detection ranges too. Indeed, during the sound recordings, only large individuals were recorded with the hydrophone placed at 100 m (Figure 19). In marked contrast, intermediate, small and very small individuals were not recorded with our hydrophones above 50, 20 and 10 m, respectively (Figure 19). These results were confirmed through the detection ranges estimated using the passive sonar equation, where intermediate, small and very small individuals can be detectable up to 100, 50 and 15 m, respectively (see Table 9). Under the ANL recorded during the experiment, large individuals are expected to be recorded at 250 m underwater with the largest spiny lobsters (CL = 13.5 cm) being detectable above 400 m (Table 9). Using a theoretical low (but still realistic) ANL (Wenz 1962), the detection ranges for large individuals were estimated at 750 m with the largest individuals being detectable up to 1080 m. In this study, the global TL calculated for spiny lobsters ranged between the theoretical models of practical and spherical TL, which are the most conservative models of TL (*i.e.* loss of 40 dB at 100 m for the spherical TL). By considering the least conservative model of TL (cylindrical), large spiny lobsters may be detectable at the kilometre scale under the theoretical ANL, with largest individuals being detected up to 3000 m (Table 9). This is consistent with a previous study which recorded broadband sounds likely produced by unknown species of invertebrates at kilometres away from coral reefs (Kaplan and Mooney 2016). This result is particularly important, because it demonstrates the possibility to use PAM for studying spiny lobsters underwater. Such studies would have a large spatial resolution, which is highly valuable for assessing their absence-presence and activity patterns in their environments, as shown for marine mammals and fish (Locascio and Mann 2008, Parks et al. 2011).

Ecological relevance

From an ecological point of view, the detection ranges discussed above may not be relevant for spiny lobsters. A more interesting quantity is the communication distance (Alves et al. 2016, Locascio and Mann 2011), i.e. the distance over which animals can “hear” each other. Communication distances are more difficult to assess because it requires additional information about the animal hearing abilities. To our knowledge, no audiogram has been performed in spiny lobsters, and nothing is known about their exact hearing sensitivity. However, it is thought that most crustaceans are sensitive to low frequency particle motion (i.e. below 1 kHz; Popper et al. 2001, Popper and Hawkins 2018). Assuming that this hypothesis holds true for spiny lobsters, our study may be used to roughly estimate the communication distances by evaluating the detection ranges of the low frequencies from antennal rasps. Nonetheless, we emphasize that only a rough estimate can be obtained since 1) we measured only sound pressure, and 2) the hearing capacity of spiny lobsters is not known. The following discussion will thus be qualitative, rather than quantitative.

The spectral content of the recorded antennal rasps was different according to the distance from the spiny lobsters. Indeed, while low frequencies (< 1 kHz) dominated close to the sound source (< 10 m), only high frequencies (> 10 kHz) remained at higher distances (> 10 m; Figures 20 and 21). Since the spiny lobsters may be sensitive to low frequencies only (< 1 kHz), the long-distance high-frequency antennal rasps are likely out of the hearing range of the animals. Thus, we can conclude that the communication distances are much shorter than the detection ranges. On the specific dataset studied here, assuming that the animals are sensitive to frequencies below 1 kHz, the communication distances would be no more than 10 m (see Figure 20), which is consistent with what is known for fish (Mann and Lobel 1997, Alves et al. 2016, Locascio and Mann 2011).

The apparent frequency shift of the antennal rasps is due to sound propagation and ambient noise, and not ecological reasons. Indeed, the ANL in the low frequencies (below 1 kHz) was 20 dB louder compared to the higher frequencies (above 10 kHz). As sounds propagate away from the source, their intensities decrease. Thus, at large ranges (above 10 m), the apparent dominant frequencies (< 1 kHz) of the antennal rasps are fully masked by the high-power low-frequency ambient noise. The recorded sounds have therefore dominant frequencies only above 10 kHz, i.e. in the frequency band where the high frequency part of the antennal rasps is not masked by the ambient noise (see Figure 21). Interestingly, another study recorded sounds produced by *Panulirus japonicus* and the authors found that the recorded antennal rasps were also dominated by high frequencies (above 10 kHz; Kikuchi et al. 2015). This study was performed in a shallow water area at depths similar as our study (between 7 and 11 m). Thus, our results show that the apparent high dominant frequencies observed at large ranges are due to acoustic masking, and likely have no ecological meanings for spiny lobsters.

In this study, we recorded sounds in very shallow waters mainly for practical reasons. However, *P. elephas* is known to inhabit coastal waters from the shore up to depths of 200 m, and large individuals are most commonly found between 50 and 100 m depths (Hunter 1999, Ceccaldi and Latrouite 2000). In addition, we recorded antennal rasps in a flat sandy bottom, while spiny lobsters tend to live in rocky habitats (Diaz et al. 2001, Goñi and Latrouite 2005). Overall, detection ranges and communication distances may change depending on the environment both because of sound propagation and ambient noise. We evaluated detection ranges by using different propagation models, and the obtained results ranged between 10 and 3000 m (depending on the size of the animals). Although simple, the models considered here represent reasonable bounds within which more realistic models may predict propagation. It is thus expected that the order of magnitude of the detection ranges evaluated in this paper hold also for other environments. On the other hand, the communication distances have been

evaluated without using propagation models. Nonetheless, the estimated values are of the same range as the water depth (i.e. 10 m). Their propagations are thus impacted little by the environment (TLs are usually modeled using simple spherical losses; Ainslie 2010). Hence, we predict that the order of magnitude of the communication distances would be similar in other environments. This must now be confirmed through the development of audiograms and the measurements of particle motion generated by the antennal rasps underwater (Goodall et al. 1990, Popper et al. 2001).

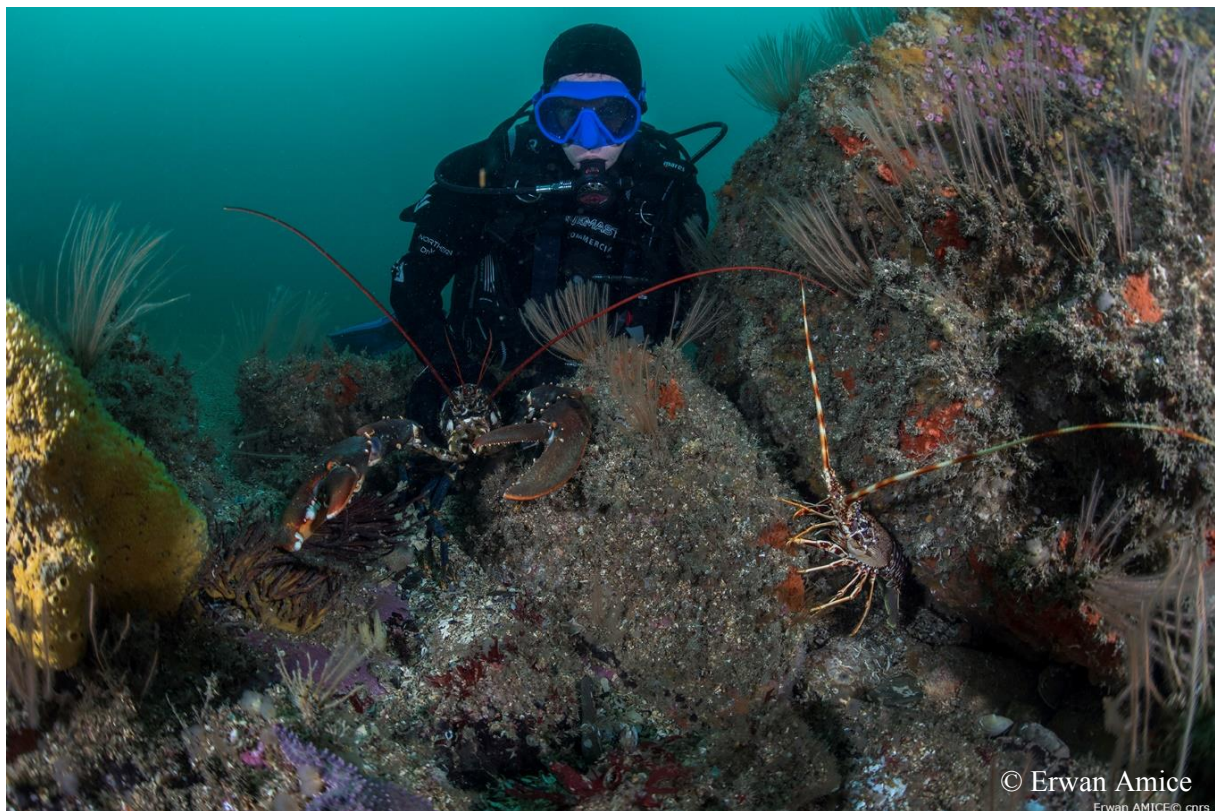
Towards a better monitoring of a vulnerable and cryptic species

In conclusion, the use of a linear array of hydrophones permitted us to examine for the first time the propagation features of the antennal rasps produced by spiny lobsters in a shallow water area. The accurate measurements of SL and TL allowed us to estimate detection ranges underwater by considering different conditions of ANL. We notably found that sounds from spiny lobsters can be detectable kilometres away underwater with hydrophones. These results will be helpful for further PAM studies because they can allow potential detection, localization and even estimation of densities of *P. elephas* over large spatial and temporal scales underwater (Rountree et al. 2006, Mann et al. 2008). The development of such non-invasive and non-destructive tools is needed to better manage this highly commercially valuable and vulnerable species that has become scarce in European coastal waters due to overfishing (Goñi and Latrouite 2005, Goñi 2014).

Acknowledgements

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Conclusion générale et perspectives



Conclusion générale

L'objectif principal de cette thèse était d'appréhender le rôle écologique des sons produits par deux espèces de crustacés, le homard Européen *H. gammarus* et la langouste rouge *P. elephas*. Nous avons également réalisé quelques expérimentations sur le homard Américain *H. americanus*. Différentes études expérimentales réalisées à la fois en laboratoire et en milieu naturel, et utilisant une grande interdisciplinarité scientifique, ont été mises en place afin de :

- Comprendre le rôle des sons produits ces deux espèces, et notamment étudier l'hypothèse d'une communication acoustique intraspécifique.
- Caractériser dans quelles mesures ces sons pourraient être utilisés comme outil de suivi *in situ* de ces populations par acoustique passive.

Les travaux issus de cette thèse ont ainsi abordé différents aspects de la bioacoustique des crustacés. Ce chapitre final se propose de résumer les principales avancées de cette thèse.

Quantification de l'impact physique des cuves sur les caractéristiques des sons produits par les crustacés

La première étape de toute étude bioacoustique impliquant des invertébrés benthiques repose sur la caractérisation de leurs sons en cuve. En se basant sur la caractérisation sonore d'*H. gammarus*, le **Chapitre 1** a démontré l'importance de prendre en compte l'effet acoustique des cuves sur les différents types de sons, qu'ils soient larges bandes (*i.e.* la plupart des sons produits par les crustacés), ou basses fréquences (*i.e.* les buzz des homards). Comprendre ces effets était une étape cruciale pour caractériser les sons enregistrés en cuve avec précision. Cette caractérisation fine est ensuite nécessaire pour détecter ces sons dans le milieu marin parmi un chorus d'autres sources sonores et/ou estimer leur potentiel rôle écologique.

Les basses fréquences, comme les buzz des homards Européens, subissent une forte atténuation car leur longueur d'onde est plus grande que la taille des cuves, ce qui rend leur détection avec des hydrophones très difficile (Jézéquel et al. 2018). En tenant compte de ce verrou acoustique, dans le **Chapitre 2**, nous avons imposé des rencontres agonistiques entre homards mâles pour caractériser leur comportement acoustique durant ces moments de fortes interactions. Afin de palier au biais induit par les propriétés physiques des cuves (i.e. l'atténuation de leurs buzz), nous avons utilisé de petits accéléromètres collés sur la carapace des animaux pour détecter, non plus les sons, mais les vibrations qui génèrent les buzz (i.e. la source sonore). Cela nous a permis de montrer que durant ces rencontres, tous les homards faisaient vibrer de nombreuses fois leurs carapaces. Cependant, les buzz associés étaient le plus souvent si atténués qu'ils n'étaient que très peu détectables par les hydrophones déployés dans la cuve. Les homards émettent donc des sons entre eux ! Ce résultat a remis au centre du débat l'hypothèse d'une communication acoustique intraspécifique chez le homard Européen pendant les rencontres agonistiques entre individus mâles.

Les buzz de homards sont des signaux à bande étroite (i.e. contenus dans une petite bande de fréquence), ce qui est plutôt rare pour les crustacés. En effet, la plupart des sons reportés chez les crustacés sont de type large bande. Lorsqu'ils sont mesurés en cuve, ils sont donc fortement impactés par 1) l'atténuation (aux basses fréquences), 2) la réverbération (aux hautes fréquences), et 3) la résonance (aux fréquences intermédiaires ; Jézéquel et al. 2018). Or, nous avons constaté que la plupart des publications de la littérature bioacoustique caractérise ces sons en cuve sans tenir compte de ces différents biais méthodologiques. C'est également le cas des rasps d'antennes des langoustes (*P. elephas*). Dans le **chapitre 4**, nous avons comparé les caractéristiques sonores des rasps d'antennes produits par *P. elephas* en cuve et dans le milieu marin. Nous montrons que les différences entre les caractéristiques sonores étaient toutes significatives, comme la durée des impulsions qui était rallongée à cause de la

réverbération des signaux en cuve. Le contraste le plus marquant était contenu dans la forme spectrale. En effet, les sons enregistrés en laboratoire étaient fortement déformés par la résonance dans les hautes fréquences (> 2 kHz), ainsi que par l'atténuation des basses fréquences (< 2 kHz). A l'inverse, les rasps d'antennes enregistrés dans le milieu marin présentaient un contenu énergétique majoritairement présent dans les basses fréquences (< 1 kHz). Puisqu'il est communément accepté que les crustacés sont capables de détecter les sons basse fréquence, ce résultat nous a également permis de proposer l'hypothèse de communication intraspécifique des antennal rasps produits par *P. elephas*. Avant ce travail de thèse, cette hypothèse n'avait jamais été proposée, car les rasps d'antennes mesurés en cuve ne présentent pas de contenu basse fréquence.

Ces différentes études en laboratoire démontrent la nécessité de caractériser précisément les sons produits par les crustacés en tenant compte des différents impacts acoustiques des cuves. Si on ignore ces biais méthodologiques, les données acquises amènent les auteurs de ces travaux à des conclusions erronées. Les résultats issus de nos travaux sont en accord avec plusieurs études récemment publiées. Novak et al. (2019) ont montré la forte atténuation des sons basses fréquences produits par des poissons, ce qui limite leur détection en cuve. De plus, nos résultats ont été repris et confirmés par deux études travaillant sur la bioacoustique des invertébrés en cuve (céphalopodes : Jones et al. 2019 ; crustacés : Filiciotto et al. 2019). Cependant, la majeure partie de la littérature bioacoustique des crustacés publiée durant la période de cette thèse ne tient pas compte de ces biais méthodologiques (e.g. Silva et al. 2018, Flood et al. 2019, Sal Moyano et al. 2019, Oka et al. 2019, Peixoto et al. 2020). Ce constat est attribué à une méconnaissance de l'acoustique en cuve par la communauté bioacoustique. Cela montre surtout l'importance d'une approche expérimentale intégrant une forte interdisciplinarité, associant les sciences de l'ingénieur et de la physique à l'écologie et biologie marine, comme cela a été mis en place durant ce projet de thèse.

La communication intraspécifique chez les crustacés : le rôle sous estimé des sons ?

Le **Chapitre 2** a clairement démontré la forte production de buzz par *H. gammarus* durant des rencontres agonistiques. Il était donc légitime de proposer l'hypothèse du rôle de ces buzz pour la communication intraspécifique. Cependant, le manque de connaissances sur les capacités auditives des homards ne permettait pas de valider aisément cette hypothèse. Dans ce contexte, nous avons effectué une étude neurophysiologique pour caractériser la détection sonore par le homard Américain *H. americanus* (**Chapitre 3**), une espèce très proche de *H. gammarus* de par son anatomie, les comportements agonistiques et les buzz qu'ils produisent. Le résultat le plus important présenté dans ce travail est sans nul doute l'établissement de l'audiogramme de cette espèce. En effet, nous avons démontré que le homard Américain est capable de détecter les sons dans les basses fréquences (entre 80 et 250 Hz), avec la meilleure sensibilité auditive entre 80 et 120 Hz. Cette plage de fréquence coïncide avec les fréquences émises lors des buzz par la même espèce. Ces nouveaux éléments confortent ainsi l'hypothèse du rôle écologique de ces buzz pour la communication intraspécifique chez les homards, et présentent un intérêt pour les études centrées sur l'impact anthropique sur cette espèce.

Le **chapitre 4** a montré que les antennal rasps produits par les langoustes dans le milieu marin présentent un contenu énergétique dominant dans les basses fréquences (< 1 kHz), qui pourrait être détectable (en théorie) par leurs congénères (Budellmann 1992, Popper et al. 2001). Dans le **chapitre 5**, nous avons estimé des distances de communication dans le milieu marin en étudiant l'évolution de ce contenu énergétique en fonction de la distance des langoustes. Nous avons pu démontrer que ce contenu énergétique dans les basses fréquences est rapidement masqué par le bruit ambiant (non détectable au-delà de 10 m). En conséquence, si les langoustes communiquent acoustiquement, elle ne peuvent le faire que sur de courtes distances (*i.e.* quelques mètres). Ce moyen de communication pourrait être facilité par leur comportement grégaire (Zimmer-Faust and Spanier 1987).

Le fort potentiel de l'acoustique passive pour suivre la langouste rouge dans son environnement

Nous nous sommes également intéressés au potentiel des sons produits par nos deux modèles d'étude pour des suivis de ces populations benthiques *in situ* par acoustique passive. Dans un contexte où s'impose le développement de nouveaux outils écologiques non invasifs et non destructeurs, en particulier pour étudier des espèces vulnérables et cryptiques, la priorité de ce travail a été donnée à la langouste rouge *P. elephas* (cf Préambule de la Partie 2).

Le **Chapitre 5** a quantifié la propagation des rasps d'antennes produits par une large gamme de taille de *P. elephas* en milieu côtier à l'aide d'une antenne linéaire d'hydrophones de 100 m de long. Nous avons pu mesurer précisément les niveaux sources des sons émis par chaque individu. Ces niveaux sont dépendants de leur taille, les grosses langoustes produisant les sons les plus intenses. Nos mesures ont également permis d'évaluer la propagation sonore (i.e. pertes de transmission), qui dépend uniquement du milieu physique et non de la source sonore. A partir de ces deux mesures, des estimations de distances de détection ont été calculées. Sans surprise les gros individus étaient détectés à une plus grande distance que les plus petits. Nous avons par ailleurs montré que les rasps d'antennes produits par les plus grosses langoustes (i.e. adultes) pouvaient être détectées à plusieurs kilomètres (!) avec des hydrophones dans le milieu marin.

D'un point de vu méthodologique maintenant, ces résultats ajoutent bien évidemment une nouvelle dimension au potentiel de l'acoustique passive pour suivre cette espèce de crustacé sur de grandes échelles spatiales. L'acoustique passive semble donc constituer un outil prometteur pour suivre les comportements et déplacements de cette espèce vulnérable, mobile et vivant dans habitats complexes et difficiles d'accès.

Perspectives

Les résultats issus de ce projet de thèse lèvent de nouvelles hypothèses en écologie marine, et proposent également de nouveaux outils pour étudier les crustacés. Ce dernier chapitre présente les perspectives de recherche qui pourraient être mises en place pour compléter et poursuivre ces travaux.

Vers la création d'un aquarium acoustiquement neutre ?

Nous avons vu tout au long de cette thèse l'importance de quantifier les impacts physiques des cuves sur les différents types de sons communément produits par les crustacés. Les différentes méthodologies présentées dans les **Chapitres 1, 2 et 4** sont applicables à toutes les espèces d'invertébrés benthiques dont on souhaiterait caractériser les sons. Dans un contexte où les études bioacoustiques sur les crustacés sont amenées à augmenter dans les prochaines années (Williams et al. 2015), nous présentons d'autres moyens pour (tenter de) réduire les impacts physiques des cuves sur les sons.

Le développement de cuves anechoïques, dont les parois sont construites de manière à absorber les ondes sonores, pourrait être envisagé. Dans cette optique, nous avons effectué différentes expérimentations à but préliminaire. Nous avons par exemple effectué des enregistrements sonores avant et après avoir recouvert les parois verticales de la plus petite cuve utilisée dans le **Chapitre 1** avec des structures en formes de nid d'abeilles. Le signal émis (i.e. chirp haute fréquence) dans la cuve avec les structures en nid d'abeilles était nettement moins affecté par la résonance et la réverbération (Figure 22 C), et ressemblait plus au signal d'origine (Figure 22 A) comparé au signal enregistré sans la structure (Figure 22 B). Cependant, nous avons décidé de ne pas utiliser ce matériel pour nos expérimentations car ses effets sur la réverbération et la résonance doivent être caractérisés complètement, ce qui dépassait le cadre de ce projet.

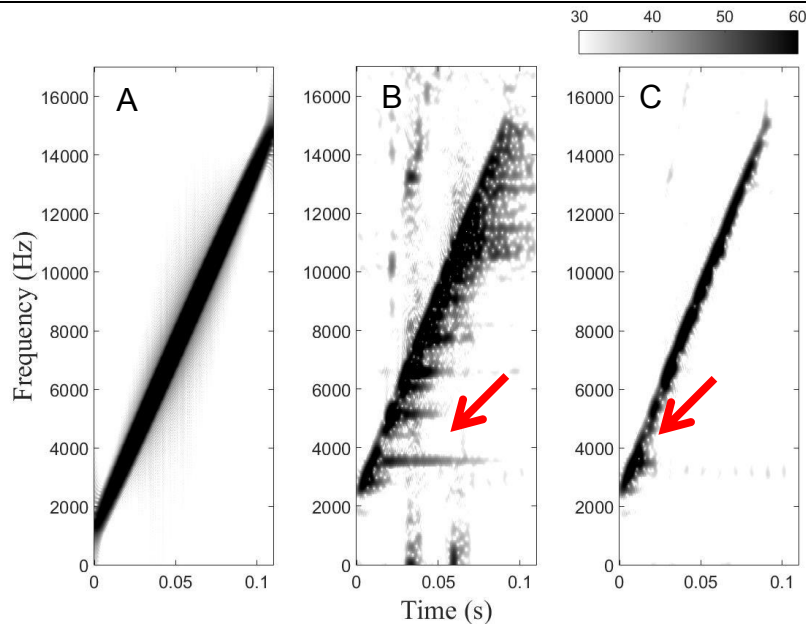


Figure 22: Spectrogrammes d'un chirp artificiel émis dans une cuve de 0.105 m^3 (cf **Chapitre 1**) et enregistré à 10 cm de l'hydrophone. A : Signal d'origine; B : Signal reçu; C : Signal reçu avec les quatre parois verticales recouvertes d'une structure en nid d'abeille. Les flèches rouges indiquent la fréquence de résonance minimale de la cuve (i.e. 3.5 kHz). L'échelle de couleur est en $\text{dB re } 1 \mu\text{Pa}^2 \text{ Hz}^{-1}$.

Notons également que ces différents enregistrements bioacoustiques en cuve sont généralement réalisés avec une interface air-eau libre, ce qui représente un changement de densité impactant fortement la propagation des sons. Nous pourrions imaginer des cuves entièrement recouvertes par des structures absorbantes, mais cela limiterait le contrôle (e.g. visuel) des expérimentations avec les modèles d'études. A notre connaissance, il n'existe pas aujourd'hui dans la littérature bioacoustique de cuves anechoiques pour effectuer de telles expérimentations. C'est pourtant une perspective importante pour les études bioacoustiques en laboratoire, illustrant parfaitement l'importance de collaborations interdisciplinaires entre biologistes et acousticiens.

D'autres outils remplaçant les hydrophones peuvent également être utilisés pour palier aux insuffisances et limites de ces derniers en cuve. C'est le cas des accéléromètres. Dans le **Chapitre 2**, nous avons montré que ces capteurs peuvent être utilisés de façon complémentaire aux hydrophones car ils mesurent des variables qui sont proximi des buzz produits par les

homards. En effet, les vibrations de leurs carapaces (qui génèrent les buzz) ne sont pas impactées par la physique des cuves. Récemment, Zenone et al. (2019) ont aussi démontré que l'utilisation des accéléromètres était pertinente pour détecter la production des antennals rasps par la langouste rouge *P. elephas* en cuve. En plus de détecter la production des sons, les accéléromètres enregistrent les mouvements des organismes à très fine échelle, ce qui peut nous renseigner précisément sur leurs comportements lors de la production des sons (cf **Chapitre 2**). Nous suggérons donc de poursuivre le développement et l'utilisation de capteurs embarqués sur les animaux pour compléter les études bioacoustiques.

Vers la confirmation de la communication intraspécifique chez le homard et la langouste ?

Aujourd'hui encore, l'idée que les crustacés pourraient utiliser des sons pour communiquer reste une idée farfelue. Les **Chapitres 2** et **3** représentent donc une réelle avancée dans ce domaine, où nous suggérons que les buzz produits par les homards sont utilisés comme une communication intraspécifique. Des travaux complémentaires doivent être réalisés afin de vérifier cette hypothèse. Des éléments statistiques ont été proposés dans le **Chapitre 2** afin de tester l'hypothèse d'une communication sonore basée sur les séquences de vibrations entre homards (en tant qu'appel et réponse). Ces analyses ayant été effectuées à des fins exploratoires, il est important de noter qu'elles sont largement à améliorer. Par exemple, nous n'avons pas tenu compte dans ces analyses du rythme et/ou des silences entre. Les travaux à venir pourraient adresser la question de la communication intraspécifique par d'autres moyens d'analyses, comme la réponse comportementale d'individus face à des buzz durant des rencontres agonistiques (e.g. Breithaupt and Eger 2002), ou encore l'intégration de nouvelles théories du langage issues de la linguistique humaine (e.g. Engesser and Townsend 2019).

De plus, nous ne pouvons pas rejeter l'hypothèse que la forte atténuation des buzz due à la cuve (cf **Chapitre 2**) ait pu engendrer leur non détection par les animaux. Cela représente

un problème inhérent à toute étude bioacoustique effectuée en cuve en général, que ce soit pour les poissons ou les invertébrés (Carroll et al. 2017, Popper and Hawkins 2018). Pour palier à ce problème, il apparaît désormais nécessaire que les prochaines études de communication soient complétées par des expérimentations en cage dans le milieu marin (i.e. milieu semi-contrôlé). Elles permettront ainsi d'obtenir les réponses des modèles d'étude face aux sons réellement propagés dans le milieu marin. Ces études présentent néanmoins de nouveaux défis à relever. C'est le cas du masquage acoustique par le bruit ambiant (voir la prochaine partie sur les bruits anthropiques ci-dessous) et de la visibilité (difficulté d'observer les comportements en milieux turbides).

Le comportement acoustique des langoustes dans un contexte intraspécifique n'est pas connu. Certaines études suggèrent l'utilisation de leurs rasps d'antennes pour menacer des conspécifiques lors de rencontres agonistiques, notamment pour l'accès à la nourriture (Berrill 1976, Mulligan and Fischer 1977). Nous avons effectué en laboratoire des expérimentations préliminaires en étudiant le comportement acoustique d'individus juvéniles de *P. elephas* (utilisés dans le **Chapitre 4**). En augmentant la densité d'individus (i.e. 1, 2, 4 puis 8) auxquels une source de nourriture limitée était proposée dans une cuve expérimentale, nous avons observé un accroissement du nombre de rencontres agonistiques associé à une augmentation de la production de rasps d'antennes (Figure 23). Nous avons pour objectif de reproduire ces expérimentations en cage dans le milieu naturel en juin 2019, mais la météo et des difficultés matérielles ont eu raison de notre pugnacité. Il serait également intéressant d'étudier les capacités auditives de cette espèce en utilisant la méthode présentée au sein du **Chapitre 3**, afin de mettre à l'épreuve nos hypothèses sur la détection de leurs rasps d'antennes dans les basses fréquences (< 1 kHz).

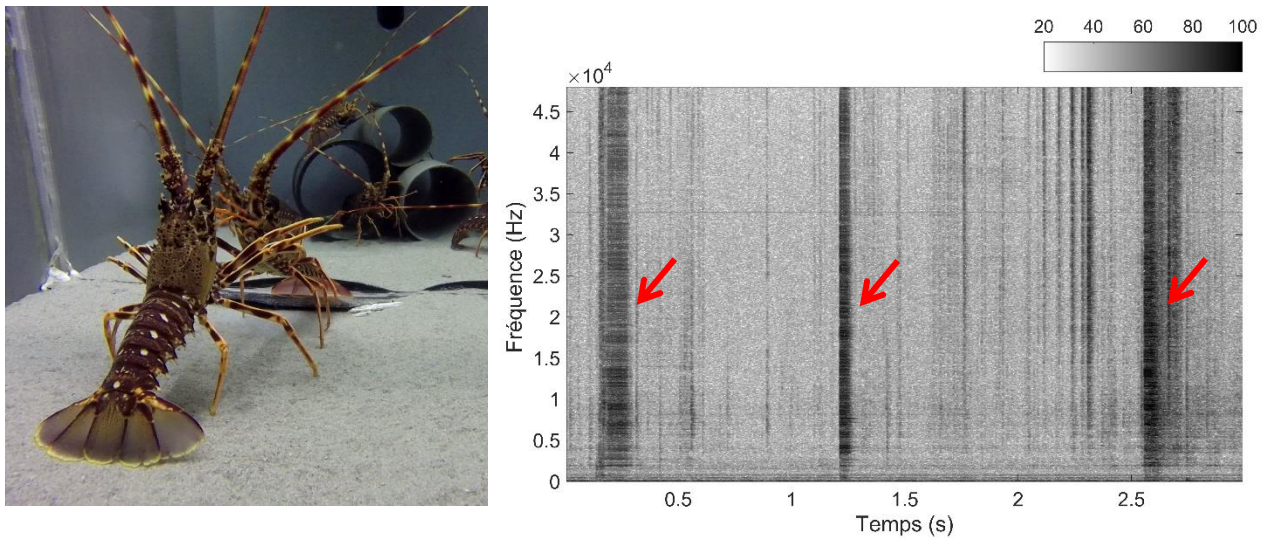


Figure 23: Photographie (gauche) d'une rencontre agonitique entre deux langoustes juvéniles (*P. elephas*) où les deux individus présentent des comportements d'intimidation physique (similaires au homard Européen dans le Chapitre 2). Durant cette rencontre, les deux individus ont produit trois raps d'antennes (flèches rouge dans le spectrogramme à droite). L'échelle de la barre de couleur est en dB re $1 \mu\text{Pa}^2 \cdot \text{Hz}^{-1}$.

Pour conclure cette partie, notons également que ces crustacés marins ont des descendants communs, les arthropodes terrestres, dont les similitudes en termes de communication semblent importantes. Nous avons vu que les différentes espèces de langoustes et de homards utilisent des signaux chimiques et visuels pour maintenir une hiérarchie sociale au sein d'un groupe d'individus (Breithaupt and Atema 2000, Shabani et al. 2009, Bruce et al. 2018). Nos études suggèrent aussi un rôle des signaux acoustiques dans ce processus. Ce type de communication multimodale est bien connu chez les arthropodes terrestres (Bell 1980, Elias et al. 2006). De nombreuses études ont par ailleurs démontré que les sons sont soumis à la sélection sexuelle pour améliorer l'émission de signaux sonores chez les insectes, ce qui est important dans l'attraction des partenaires pour la reproduction (Simmons and Ritchie 1996, Robinson and Hall 2002). Les futures études se focalisant sur ces différents types de communication chez les crustacés devront se faire en rapport étroit avec cette littérature déjà disponible chez les arthropodes terrestres (Podos and Patek 2015). Cela permettra notamment de mieux comprendre l'intérêt, d'un point de vue évolutif, du rôle des sons pour la communication intraspécifique.

Vers des études d'impacts des bruits anthropiques sur les crustacés

La communication intraspécifique chez les crustacés représente un enjeu de recherche majeur pour les prochaines années à venir. En effet, ce sont ces études qui vont permettre de quantifier précisément les potentiels impacts des bruits générés par les activités humaines sur leurs comportements. En effet, nos résultats issus des **Chapitres 2 et 3** démontrent l'utilisation potentielle des buzz chez les homards (*H. gammarus* et *H. americanus*) pour communiquer entre individus. L'anthropophonie dominant essentiellement les basses fréquences (< 1 kHz), incluant les buzz des homards, nous pensons qu'il y a de réelles inquiétudes sur les impacts potentiels de ces bruits sur ces deux espèces. Au cours de la dernière décennie, il y a eu un intérêt croissant pour la production d'énergie renouvelable marine en mer, ce qui a entraîné une augmentation de l'activité de battage de pieux dans de nombreuses régions océaniques du monde (Bailey et al. 2014). Cette activité est une préoccupation majeure compte tenu de la forte intensité qu'elle génère sous l'eau (i.e. au-dessus de 200 dB re 1 μPa^2 en pic-pic à 300 m de la source sonore ; Dahl et al. 2015). Il est donc aujourd'hui nécessaire de développer de nouveaux outils permettant de quantifier la compatibilité entre le développement de ces activités humaines et la conservation des populations de crustacés dans des directives de développement durable.

Dans ce contexte, nous avons soumis en début d'année une proposition de projet de recherche à la National Lobster Sea Grant (NOAA, Etats Unis), pour un contrat de post doctorat en collaboration avec Aran Mooney au Woods Hole Oceanographic Institution (cf **Chapitre 3**). La première partie de ce projet vise à étudier l'impact du bruit de battage de pieu sur le comportement de *H. americanus* en cuve. En effet, plusieurs zones ont été établies sur la côte Est des Etats Unis pour la création de nouveaux parcs éoliens (Beiter et al. 2018). Or, ces zones correspondent également à l'habitat de *H. americanus*, qui constitue la pêche la plus importante du pays (Maine DMR 2018). Les différentes méthodologies en cuve déployées lors

de ce projet de thèse sont susceptibles de quantifier précisément ces impacts sur le comportement des homards. Par exemple, nous avons vu dans les **Chapitres 1 et 2** que l'intensité sonore dans une cuve dépend fortement de la configuration source-receveur. Ainsi, l'animal étudié pourrait montrer des niveaux de réponse différents selon son emplacement dans la cuve, donc à cause des spécificités de la cuve et non pour des raisons biologiques. Il sera ainsi important d'effectuer des mesures précises du champ sonore en cuve avant toute exposition aux bruits (Jones et al. 2019, 2020). Nous étudierons également l'impact de ces bruits sur les jeunes stades juvéniles qui pourraient être plus affectés comparé aux adultes. En effet, la transition entre la phase de vie larvaire pélagique et la métamorphose en juvénile benthique est sensible, et représente une étape importante dans le recrutement des populations (Wahle and Incze 1997; Cowan et al. 2001).

Les bruits liés au trafic maritime sont également de réelles préoccupations pour les invertébrés benthiques. En effet, ils constituent la source sonore d'origine anthropique la plus commune dans les océans. C'est le cas de la rade de Brest qui est connue pour ses nombreuses activités marines récréationnelles, commerciales et militaires, qui génèrent un bruit important dans cette zone (Mathias et al. 2016, Kinda et al. 2017). Un des principaux impacts de ce bruit est le masquage acoustique, qui a été largement démontré chez les mammifères marins et les poissons (Clark et al. 2009, Radford et al. 2014). Nous avons également effectué des enregistrements sonores préliminaires dans la Baie de Sainte Anne (même site d'étude que dans le **Chapitre 5**) en juin 2019 lors de rencontres agonistiques en cages entre homards Européens mâles, selon le protocole établie dans le **Chapitre 2**. Tandis que les accéléromètres ont détecté de nombreuses vibrations produites par les individus, leurs buzz associés étaient peu enregistrés par les hydrophones (Figure 24). Cette différence est liée au masquage des buzz par le bruit ambiant basse fréquence (< 1 kHz) largement dû aux bruits de bateau lors des expérimentations (Figure 24). Nous avons pu mettre en évidence, de façon préliminaire, le masquage acoustique

chez les homards. Ces résultats suggèrent que les individus ne seraient pas capables de s'entendre lors des passages de bateau, ce qui pourrait affecter leur comportement.

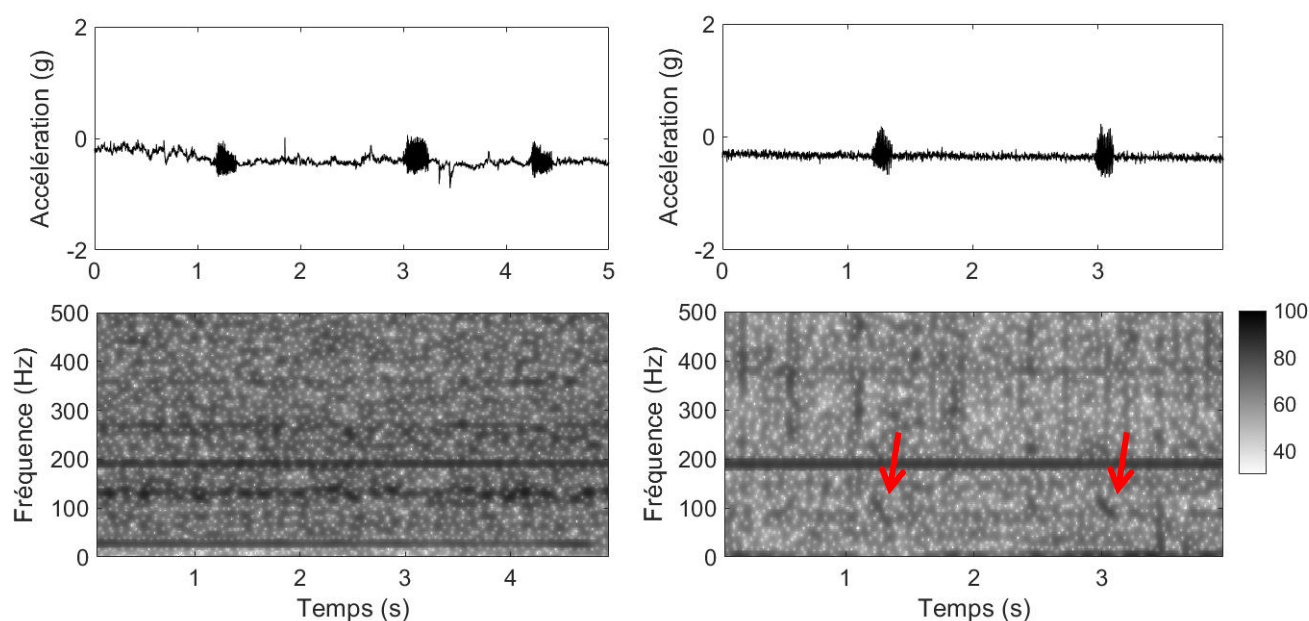


Figure 24: Données synchronisées d'un accéléromètre collé sur la carapace d'un homard (en haut) et d'un hydrophone (en bas) durant une rencontre agonistique entre deux homards mâles Européens en cage à Sainte-Anne du Portzic le 23 avril 2019. Deux enregistrements sont représentés : lors d'un passage de bateau (à gauche) et avec le bruit ambiant naturel (à droite). Notez que les buzz (flèches rouges) associés aux différentes vibrations produites par le homard dominé ne sont pas détectés lorsque le bruit du moteur de bateau est présent sur les enregistrements sonores (en bas à gauche). L'échelle de la barre de couleur est en dB re $1 \mu\text{Pa}^2 \cdot \text{Hz}^{-1}$.

Ce masquage constitue également un véritable défi acoustique pour pouvoir détecter les sons basses fréquences produits par les homards dans le milieu marin. Dans ce contexte, la deuxième partie du projet soumis à la National Lobster Sea Grant s'intéressera à l'utilisation de l'acoustique passive pour détecter les buzz *in situ*. En considérant les effets du bruit ambiant dans les basses fréquences, nous quantifierons leur propagation sonore (avec le même protocole que dans le **Chapitre 5**) dans un milieu naturel fermé, d'une surface de plusieurs kilomètres carré. Cet ancien vivier, historiquement utilisé par les pêcheurs pour stocker leurs homards, nous permettra de caractériser les buzz avec un bruit ambiant naturel, c'est-à-dire non impacté par les bruits d'origine humaine. Cela représentera une première étape vers l'utilisation de l'acoustique passive pour suivre les homards *in situ*.

Vers le développement de nouveaux outils pour suivre les crustacés dans leur environnement par acoustique passive

L'écologie marine est à la recherche de nouveaux descripteurs de l'état de santé des populations d'organismes marins, notamment des espèces vulnérables. Dans ce contexte, notre étude présentée dans le **Chapitre 5** démontre une opportunité réelle pour utiliser l'acoustique passive comme nouvel outil d'étude pour suivre la langouste rouge *P. elephas* dans son environnement en écoutant les sons qu'elle produit. En effet, nous avons démontré que les sons produits par les individus adultes peuvent être détectés sur plusieurs kilomètres avec des hydrophones dans le milieu marin. La détection et la localisation de ces reproducteurs représentent un enjeu majeur en sciences halieutiques car ils déterminent les capacités de renouvellement des populations. L'aide de nouvelles technologies, telle que l'acoustique passive, constitue donc un véritable atout dans leurs mesures de gestion.

De plus, de récentes observations relatent la recrudescence des populations bretonnes de langouste rouge depuis 2013 (Cotten 2016). Nous sommes donc dans une période charnière et devons urgemment approfondir nos connaissances sur l'écologie de cette espèce pour établir de nouvelles mesures de gestion durable. Nous avons ainsi effectué, en collaboration avec le club de plongée d'Audierne, une étude d'acoustique passive (test préliminaire) durant trois nuits consécutives dans la Baie d'Audierne en Septembre 2019. Nous avons placé un hydrophone sur une zone où nous avons observé en plongée plus d'une dizaine de langoustes juvéniles par 30 m de profondeur. Les résultats sont prometteurs, puisque malgré une durée d'enregistrement courte, nous avons pu détecter une dizaine de rasps d'antennes de langoustes durant la première nuit (Figure 25).

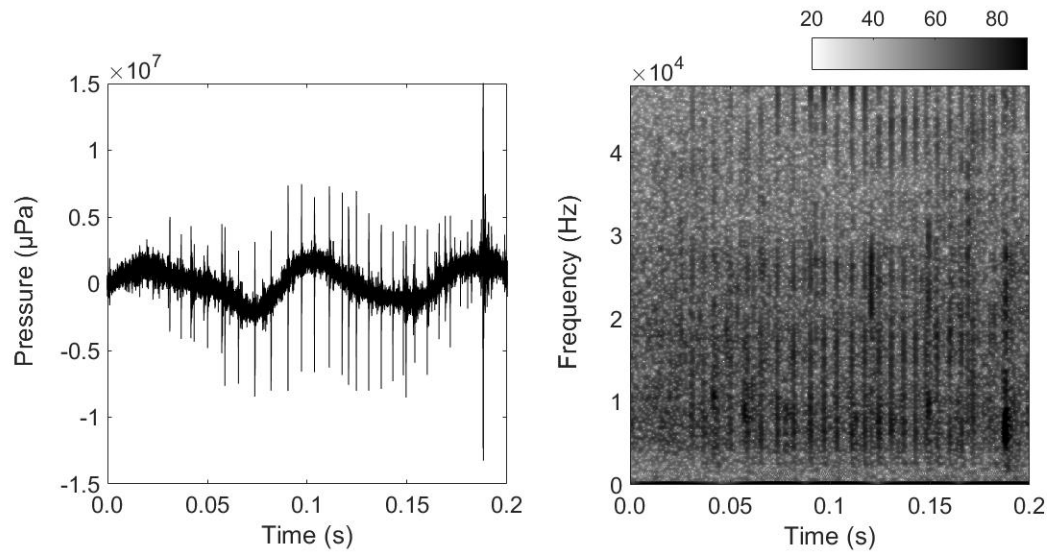


Figure 25: Forme d'onde (gauche) et spectrogramme (droite) d'un rasp d'antenne enregistré dans la baie d'Audierne durant la nuit du 13 au 14 septembre 2019. L'échelle de la barre de couleur est en dB re $1 \mu\text{Pa}^2 \cdot \text{Hz}^{-1}$.

Il convient de noter que débiter des études d'acoustique passive sur une population aussi relictuelle n'est pas chose aisée. En effet, on ne connaît ni les comportements acoustiques naturels, ni les périodes de production sonore de ces crustacés. Quand les langoustes produisent-elles leurs rasps d'antennes ? Quelle est la fréquence de production sonore par individu ? Cette production sonore est-elle différente ou similaire entre une population en bonne santé et une population impactée par la surpêche ? Peut-on estimer la taille des individus entendus en fonction de leurs caractéristiques sonores ? Ces différentes questions montrent les zones d'ombre qu'il reste à éclaircir avant d'utiliser l'acoustique passive pour suivre cette espèce. Par exemple, si les langoustes produisent leurs rasps d'antennes lors de rencontres agonistiques, comme nous avons pu le voir précédemment, il est possible que de faibles densités d'individus ne leur permettent pas de se rencontrer régulièrement, générant donc peu de sons. Il serait alors intéressant de faire des enregistrements au niveau de populations en bonne santé (*i.e.* présentant de fortes densités d'individus), comme chez les langoustes tropicales des Caraïbes ou de Californie (*Panulirus argus*, *P. interruptus* ; Phillips 2008). Ces enregistrements pourraient

nous servir d'états de référence pour les comparer aux populations relictuelles impactées par la surpêche, comme *P. elephas* en Europe.

La langouste rose *P. mauritanicus* est la deuxième espèce de langouste vivant en Europe, mais son habitat se situe à plusieurs centaines de mètres de profondeur (niche bathymétrique différente de *P. elephas* ; Goni and Latrouite 2005). Comparé à la langouste rouge, l'état de nos connaissances sur l'écologie de *P. mauritanicus* est quasi-nul. En effet, bien qu'elle ait fait la richesse de nombreuses pêcheries françaises, notamment dans les eaux mauritaniennes, la langouste rose a été décimée par la surpêche à la fin du 20^{ème} siècle (Pencalet-Kerivel 2008). Nous avons eu la chance durant cette thèse d'enregistrer les sons d'individus *P. mauritanicus* dans les viviers d'Audierne en 2018, et leurs rasps d'antennes paraissent similaires à ceux de *P. elephas* (*i.e.* trains d'impulsions larges bandes). Il apparait donc envisageable d'utiliser aussi l'acoustique passive pour détecter cette espèce cryptique et vivant dans un habitat difficile d'accès. Dans ce contexte, les langoustes pourraient ainsi devenir de nouvelles espèces sentinelles de l'environnement pour caractériser, par exemple, un impact mécanique d'origine humaine telle que la surpêche.

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Annexes

Annex 1. Supplement Table summarizing the data on the characterization of antennal rasps for spiny lobsters. Results given in these studies are presented as mean \pm SD, when available. When the dimensions of the tanks used during the sound recordings were available, we estimated their minimum resonant frequencies using the equations of Akamatsu et al. (2002). Note that these are only estimates, because the effective heights of the tanks used are not always given by authors. They were used to compare with spectral features from the articles when available. NM: not mentioned. NA: not applicable. TP: technical problem due to tank reverberation. To be complete, we also added intensity features (SPL in dB; denoted with *) from Latha et al. (2005), Patak et al. (2009), Buscaino et al. (2011) and de Vicenzi et al. (2015). Unfortunately, these results are not comparable because they were obtained with different signal processing methods and the authors did not provide enough information to be able to re-estimate the values.

Antennal rasp features

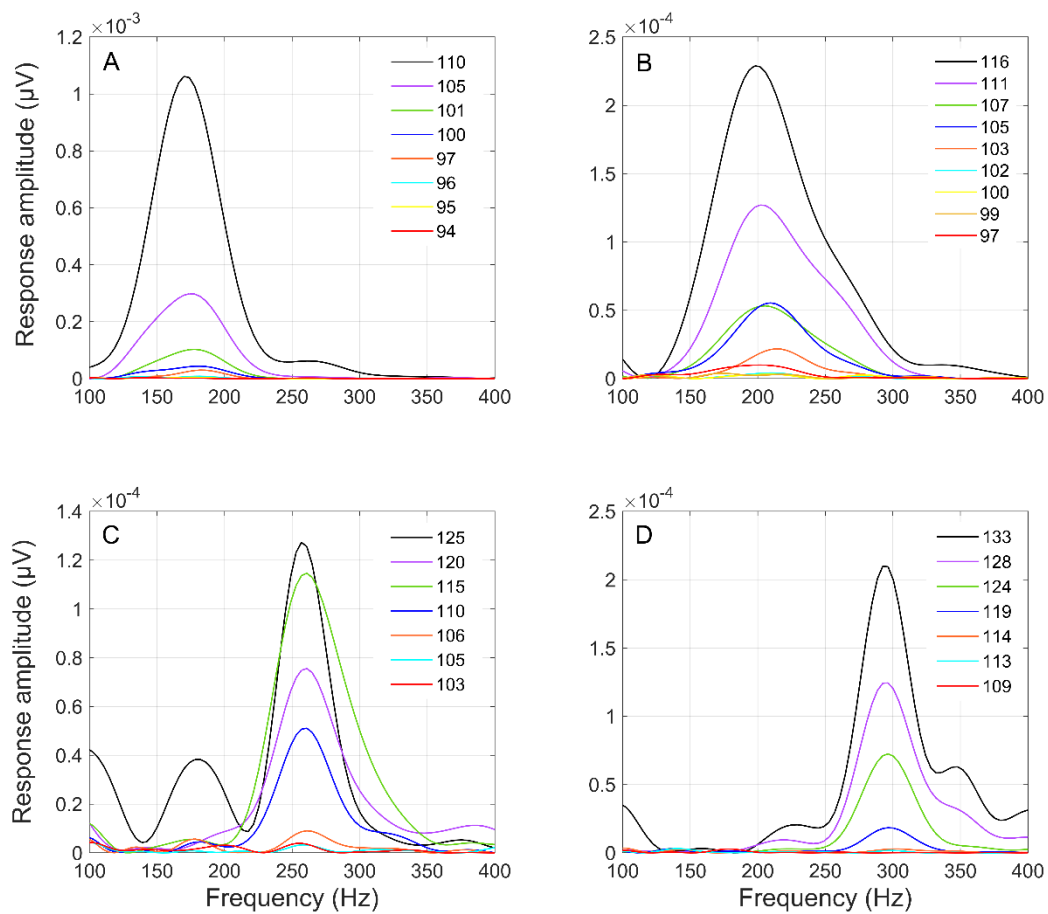
Reference	Species	Number of individuals	Carapace length (cm)	Site of sound recordings (m)	Distance from hydrophone (cm)	Protocol for eliciting antennal rasps	Antennal rasp features		Estimated minimum resonant frequency (kHz)	1 st peak Frequency (kHz)	2 nd peak Frequency (kHz)	Bandwidth (kHz)				
							Total duration (ms)	Single pulse duration (ms)								
Moulton 1957	<i>P. argus</i>	NM	NM	Large naturalized aquarium	NM	Hand held	100	NM	NM	Range 56-133	NM	NM	NA (tank dimensions NM)	0.80	2.50-4.70	Range 0.04-9.00
Hazlett and Winn 1962a	<i>J. longimanus</i>	2	9	Tank (0.5×0.5×5)	15	NM	55.1 (range 35.3-113.7)	NM	NM	NM	NM	NM	2.13	Range 1.20-2.40	NM	Range 0.09-12.00
Hazlett and Winn 1962b	<i>P. guttatus</i>	1	18.9	Tank (0.5×0.5×9)	15	Handheld	53.3 (range 26-72)	NM	NM	NM	NM	NM	2.12	Range 1.20-2.40	NM	Range 0.09-12.00
Meyer-Rochow and Penrose 1974	<i>P. argus</i>	NM	Range 6.4-10	Tank (0.5×0.5×9)	15	Handheld	92.6 (range 63-111)	NM	NM	NM	NM	NM	2.12	Flat from 0.09 to 4.80	NM	Range 0.09-12.00
Meyer-Rochow and Penrose 1974	<i>P. longipes</i>	NM	Puerulus	Tank (0.5×0.5×4)	5	Handheld	20	NM	NM	NM	NM	NM	2.83	NM	NM	Range 0.09-6.00
Meyer-Rochow and Penrose 1976	<i>P. longipes</i>	NM	Post puerulus larvae	Tank (0.5×0.5×0.4)	5	Handheld	70	NM	NM	NM	NM	NM	2.83	Range 0.10-1.00 and 2.00-3.00	Range 5.00-7.00	Range 0.09-11.00
Meyer-Rochow and Penrose 1976	<i>P. longipes</i>	NM	Range 1-10	Tank (0.5×0.5×0.5)	5	Handheld	Range 60-220	NM	NM	Range 9-34	NM	NM	2.83	TP	TP	TP

Mulligan and Fischer 1977	<i>P. argus</i>	25	NM	Tank (0.26m ³)	NM	Agonistic interactions	153 ± 54.7 (range 84-288)	22 ± 1.4	23 ± 1	6.6	NM	NM	NM	N/A (tank dimensions NM)	2.30	3.40	Range 0.08-16.00	
Patek 2001	<i>P. argus</i>	6	NM	NM	NM	NM	NM	NM	NM	NM	77	NM	NM	NM	NM	NM	NM	
Patek 2002	<i>P. argus</i>	6	Range 4.7-9.7	Tank (1.32×0.79×0.64)	15	Handheld	103.7 ± 5.7 (range 19.2-331.6)	1.7 ± 0.03 (range 0.6-13)	6.9 ± 0.4 (Range 2-24)	76.7 ± 3.2 (range 24.1-218)	NM	NM	NM	NM	NM	NM		
Patek and Oakley 2003	<i>P. elephas</i>	2	NM	NM	15	Handheld	101 ± 45.7	NM	13.5 ± 5.4	138.5 ± 40.5	NM	NM	NM	NM	NM	NM		
	<i>P. wagensis</i>	1	NM	NM	15	Handheld	67.4 ± 28.6	NM	8.9 ± 4.5	129.4 ± 15.8	NM	NM	NM	NM	NM	NM		
	<i>P. japonicus</i>	1	NM	NM	15	Handheld	155.1 ± 51.6	NM	9.5 ± 5	61.4 ± 21	NM	NM	NM	NM	NM	NM		
	<i>J. japonica</i>	1	NM	NM	15	Handheld	69.2 ± 47	NM	8.2 ± 3.8	131.4 ± 23.4	NM	NM	NM	NM	NM	NM		
	<i>L. trigonus</i>	1	NM	NM	15	Handheld	156.6 ± 42.7	NM	17.9 ± 4	118.5 ± 31	NM	NM	NM	NM	NM	NM		
	<i>P. homurus</i>	1	NM	NM	15	Handheld	89.5 ± 83	NM	9.9 ± 8.6	120.6 ± 19.8	NM	NM	NM	NM	NM	NM		
	<i>P. longipes</i>	1	NM	NM	15	Handheld	101 ± 66.5	NM	11.4 ± 7.8	147.4 ± 17	NM	NM	NM	NM	NM	NM		
	<i>P. argus</i>	4	NM	NM	15	Handheld	151.7 ± 82.3	NM	7.2 ± 1.5	65.9 ± 27.4	NM	NM	NM	NM	NM	NM		
Latha et al. 2005	<i>P. homurus</i>	NM	NM	Tank (4.3×1.3×1m)	< 50	NM	NM	NM	NM	NM	NM	NM	NM	Range 50.1-69.5 (peak amplitude) *	26.10	52.60	Range 3.00-100.00	
	<i>P. wagensis</i>	3	NM	Tank (4.3×1.3×1m)	< 50	NM	NM	NM	NM	NM	NM	NM	NM	Range 119.1-143.2 (peak amplitude) *	3.30	52.12	Range 3.00-75.00	
Patek and Bato 2007	<i>P. interruptus</i>	5	8.5-8.8	NM	NM	NM	NM	7.9 ± 2 (range 1.4-19.9)	4.5 ± 0.9 (range 3-7)	87.6 ± 15.9 (range 55.4-136.7)	NM	NM	NM	NM	NM	NM		
Patek et al. 2009	<i>P. interruptus</i>	19	4.4-10.2	Tank (1.5×0.8)	60	Handheld	108 ± 35 (range 15-303)	TPP	7 ± 3 (2-19)	71.2 ± 20 (24 - 192)	NM	NM	NM	NM	NM	NM		
	<i>P. interruptus</i>	20	50-113	Tank (1.5×0.8)	Range 31-66	Handheld	NM	NM	NM	NM	NM	NM	NM	NM	1.012	1.79±0.34	1.80±0.303	NM
	<i>P. interruptus</i>			<i>In situ</i>	31	Handheld	NM	NM	NM	NM	NM	NM	NM	NA	0.63±0.37	1.59±0.48	NM	

Buscanno et al. 2011	<i>P. elephas</i>	25	18.3 ± 2.6 (total length)	Tank	NM	Predator interactions	90±50	TPP	NM	9.5±4.5	118.2±54.4	119.8±8.4 (peak amplitude) *	NM	N/A (tank dimensions NM)	19.52±6.70	NM	122.90±23.69
	<i>P. interruptus</i>	13	65-93	<i>In situ</i>	Range 91-150	Handheld	NM	NM	NM	NM	NM	150.4±2 (peak amplitude) *	NM	NM	NM	NM	
Kikuchi et al. 2014	<i>P. japonicus</i>	NM	NM	<i>In situ</i>	NM	<i>In situ</i> monitoring	40±20	NM	4±2	11.09±4.10	NM	NM	NM	N/A	9.99±4.47	10.20±4.42	0.75±0.46
	<i>P. elephas</i>	40	7.9±0.5	Tank (2.35×1.5)	NM	Response to sounds	70±20	NM	NM	8±3.5	134±37.1	124.6±6.3 (peak amplitude) *	NM	0.56	22.93±8.20	NM	127.20 ± 33.20

Jéréquel et al. 2019	<i>P. elephas</i>	13	5.3±0.8 (range 4.2-7)	Tank (1.13×0.73×0.5)	Range 20-30	Handheld	120.5±26.0 (range 60-225)	TPP	NM	15.0±3.3 (range 7-28)	127.9±21.1 (range 78.4-226.7)	171.0±3.1 (range 160.4-175.7) (peak-peak)	151.2±4.2 (range 139.7-159.6) (rms)	NA	1.94	3.99±3.68 (range 1.82-17.74)	5.34±4.27 (range 1.82-17.83)	5.13±2.51 (range 0.42-11.70)
	<i>P. elephas</i>	9	5.0±0.3 (range 4.1-5.4)	<i>In situ</i>	Range 20-50	Handheld	147.0±29.7 (range 53-266)	NM	NM	16.9±4.7 (range 6-33)	115.9±27.2 (range 59.4-208.9)	167.3±3.9 (range 156.0-175.7) (peak-peak)	139.2±3.0 (range 132.0-146.4) (rms)	Range 154.2-160.6 (peak-peak)	NA	0.77±0.24 (range 0.12-1.66)	0.96±0.40 (range 0.22-1.62)	16.99±5.38 (range 4.90-23.00)

Annex 2. Supplementary Figure of Chapter 3



FFT frequency spectra of lobster AEPs. Responses were generated from four different tone-pip frequency stimuli: 80 Hz (A), 100 Hz (B), 120 Hz (C) and 150 Hz (D). The colors refer to the stimulus levels (SPL_{rms} , in dB re 1 μPa) presented in the legend of each graph. Note that the response amplitudes decrease as stimulus levels decrease. Similar to fish and other marine invertebrates for which AEP responses have been measured, the frequencies of the AEP responses corresponded to about twice the stimulus frequencies.



FEATURE ARTICLE

Sound characterization of the European lobster *Homarus gammarus* in tanks

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ABSTRACT: Experiments in marine behavioural ecology rely heavily on observations made in tanks. However, when studying acoustic behaviours of marine animals in confined volumes, the effects of reverberation must be characterized, something that has been overlooked in parts of the marine ecology literature. In this study, we characterized reverberation in tanks using an artificial sound source and examined the implications for bioacoustic studies using sounds emitted by the European lobster *Homarus gammarus* during feeding and in response to stress. Broadband and transient sounds commonly produced by crustaceans were severely impacted by reverberation such that their spectral characteristics and pulse width durations could not be assessed. In contrast, low-frequency sounds could be characterized in tanks, but not their source level. Based on these observations, we describe a simple methodology to identify which sound characteristics can be measured in tanks. When feeding, the lobsters produced broadband and transient sounds called 'rattles', similar to sounds reported for tropical spiny lobsters *Palinurus longipes* and *P. argus*. When stressed, *H. gammarus* vibrated its carapace, producing a low-frequency sound analogous to the 'buzzing' sound of the American lobster *H. americanus*. The potential role of species-specific sound is discussed; however, although our observations represent the first bioacoustic characterization of *H. gammarus*, additional behavioural studies are necessary to understand their ecological meaning.

KEY WORDS: European lobster · Passive acoustics · Tanks · Reverberation · Rattle · Buzzing sound · Spectral analysis



Bioacoustic experiment conducted in the Bay of Brest (Brittany, France) with European lobsters *Homarus gammarus*.

Painting:
Nathalie Bihan (redrawn from a photo by Erwan Amice)

INTRODUCTION

Bioacoustic studies of crustaceans have recently been receiving more attention in marine ecology (e.g. Edmonds et al. 2016). This has been driven partly by the commercial value, ubiquitous distribution and apparent ease of study of crustaceans compared with larger, highly mobile mammals. However, while the purpose of sounds emitted by whales and dolphins has been investigated for decades (Tyack & Clark 2000), little is known about the ecological

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roles of sounds made by crustaceans (Coquereau et al. 2016a,b, Edmonds et al. 2016). Recent studies have suggested that the sounds emitted by temperate marine decapods have properties suitable for *in situ* bioacoustic studies (e.g. *Maja brachydactyla*, Coquereau et al. 2016a).

Generally, marine crustaceans are known to produce a variety of sounds through different mechanisms, ranging from 'stridulation' in crabs (Guinot-Dumortier & Dumortier 1960, Boon et al. 2009) to 'cavitation bubble collapse' in snapping shrimps (Knowlton & Moulton 1963, Versluis et al. 2000), 'stick and slide friction' in palinurids (Meyer-Rochow & Penrose 1976, Patek 2001) and 'carapace vibration' in nephropids and stomatopods (Henninger & Watson 2005, Patek & Caldwell 2006). Most reported sounds in the bioacoustic literature on crustaceans are broadband and transient (Au & Banks 1998, Patek et al. 2009, Coquereau et al. 2016a,b). Authors have suggested many hypotheses about the roles of these sounds, including anti-predator defence in palinurids (Bouwma & Herrenkind 2009, Buscaino et al. 2011) and intraspecific communication in paddle crabs (Buscaino et al. 2015). Some sounds are described as a consequence of identifiable activities, such as the 'rattles' emitted while feeding in palinurids (Moulton 1957, Meyer-Rochow & Penrose 1976). A few crustacean species, including the American lobster *Homarus americanus* (Milne Edwards 1837), have been reported to emit low-frequency and narrowband sounds (Fish 1966, Henninger & Watson 2005). When threatened or handled, the carapace of *H. americanus* vibrates and leads to a 'buzzing sound', due to the contraction of internal muscles located at the base of the second antenna (Fish 1966, Mendelson 1969, Henninger & Watson 2005). The European lobster *H. gammarus* (Linnaeus 1758), which is closely related to the American lobster, has a similar anatomical morphology (Holthuis 1991), but no studies have yet reported on the sounds emitted by this species.

Most of the bioacoustic studies mentioned above have been performed in tanks because this permits the visual observations necessary to associate sounds with precise behaviours (Hazlett & Winn 1962a,b, Meyer-Rochow & Penrose 1974, 1976, Mulligan & Fischer 1977, Patek & Caldwell 2006, Patek & Baio 2007, Patek et al. 2009, Buscaino et al. 2011, 2015, Coquereau et al. 2016a,b). However, the sound field in a tank is highly complex because of the interference caused by multiple reflections on the tank walls. Usually there are so many reflections

that individual echoes cannot be resolved. This phenomenon is called reverberation and can be seen in data as the persistence of sound after its emission stops. It can prevent animal sounds from being properly characterized in tanks (Parvulescu 1964, 1967, Akamatsu et al. 2002). When reverberation occurs, standing waves may be generated by the superposition of reflected sound waves. Thus, what is recorded may correspond to the resonant frequencies of the standing wave because of its longer duration relative to the biological sound that was emitted (Akamatsu et al. 2002). The recorded spectrum becomes distorted and difficult to characterize. A large body of acoustic literature exists on the effects of reverberation when measuring broadband sounds (e.g. Pierce 1981, Schroeder 1996) but appears to have been largely overlooked within the bioacoustic community, although highlighted in the 1960s (Parvulescu 1964, 1967). However, the problem with reverberation has recently gained interest in this field in acoustic pressure (Akamatsu et al. 2002) and particle motion measurements (Duncan et al. 2016, Popper & Hawkins 2018).

Nevertheless, bioacoustic studies in small tanks can still provide reliable information. Recently, Akamatsu et al. (2002) described for the first time the sound distortions produced in small tanks by combining empirical approaches with calculations from acoustic theory. This important, fundamental article focused on low-frequency and narrowband sounds emitted by fish. As reverberation is highly dependent on the frequency of interest with respect to the tank's resonant frequencies (which in turn depends on the tank's dimensions), the following applies: if the considered sound has a frequency on the order of, or higher than, the tank minimum resonant frequency, it will be affected by reverberation. The sound's duration is extended and its frequency content may be altered. On the contrary, if the considered sound has a frequency largely below the tank minimum resonant frequency, then the recorded sound is not impacted by reverberation (its duration and frequency content not being altered).

The purpose of this study was to characterize sounds produced by *H. gammarus* individuals during different activities and compare them with published information on *H. americanus* and other decapods. But, considering the limits of small tanks for bioacoustic studies, the first step was to determine the conditions under which reliable information on sounds emitted by this species can be collected.

MATERIALS AND METHODS

All laboratory experiments were carried out at the Océanopolis public aquarium in Brest (Brittany, France).

The lobster *Homarus gammarus* (Crustacea, Malacostraca, Nephropidae) is a large (up to 6 kg), mobile, nocturnal and commercially important crustacean in European coastal waters (Smith et al. 1998). Its life cycle is typical for a benthic crustacean, with a pelagic larval stage followed by benthic juvenile and adult stages and growth occurring through successive periods of molts (Cobb & Wahle 1994, Sheehy et al. 1999, Agnalt et al. 2007).

Animal collection, housing and care

Seventeen *H. gammarus* individuals (10 females and 7 males) with a carapace length (CL) between 8.7 and 12 cm were collected through snorkeling in the Bay of Plougonvelin (Brittany, France) at depths between 1 and 10 m during January and February 2017. All individuals were transferred to a shaded, outdoor polyester circular tank (radius = 4 m, effective height = 1.13 m; seawater volume = 14.2 m³) for holding. The tank was continuously supplied with sand-filtered, UV-sterilized seawater pumped from the Bay of Brest. Temperature, salinity and animal conditions were controlled daily. During experiments, temperature varied between 9 and 12.5°C and salinity was between 32.8 and 34.6. Animals were fed with frozen squid, frozen mackerel and fresh mussels *ad libitum* and were kept under the natural photoperiod in this tank. Abundant sections of rigid PVC drainage pipes were provided as shelters. Lobsters were held together in the tank during the entire period of the study (around 4 mo, depending on the date of capture) when they were not being used for the experiments. No lobsters were harmed during the study. At the end of the study, all lobsters were transferred to the Océanopolis public aquarium.

Experimental tanks

Three types of rectangular tanks were used: 6 identical glass tanks (0.60 × 0.50 × 0.35 m, length × width × effective height; 0.105 m³), 1 plastic tank (1.14 × 0.92 × 0.45 m; 0.47 m³) and 1 larger polyester tank (2.10 × 2.10 × 0.53 m; 2.34 m³). During experiments, tanks were continuously supplied with the same seawater flow as for the holding tank. The 6

glass tanks and the plastic tank were in a slightly warmer room than the polyester tank, which resulted in somewhat warmer water conditions in the glass and plastic tanks (13.9–14.5°C) than in the polyester tank (9.0–12.5°C) during the study.

Recordings

Sounds were recorded using a pre-amplified hydrophone (HTI-92-WB, High Tech) with a sensitivity (SH) of −155 dB re 1 V μPa^{−1} and a flat response from 2 to 50 kHz. The hydrophone was connected to a compact autonomous recorder (EA-SDA14, RTSys) powered by battery to limit electronic self-noise. Recordings were made with a sampling frequency (F_s) of 156 kHz at 32-bit resolution. Sounds, $S(t)$, were recorded in volts and then converted to pressure, $p(t)$ in μPa, in the time-domain (t), using the following equation:

$$p(t) = S(t) \times 10^{\frac{G}{20}} \times D \times 10^{\frac{SH}{20}} \quad (1)$$

where G (dB) is the recorder gain (here $G = 14.7$ dB), D is a constant for the dynamic response of the recorder (2 V for this model) and SH is the sensitivity of the hydrophone.

Finally, to associate a sound with a particular behavioural event, both visual observations and video recordings (GoPro® HERO3 camera) were made during experiments.

Reverberation in the experimental tanks

To quantify distortion in the 3 types of experimental tanks (0.105 m³ glass tank, 0.47 m³ plastic tank and 2.34 m³ polyester tank), an artificial sound was emitted into each one with an omnidirectional underwater speaker (AQUA 30, DNH, 8 Ohms, 20–20 000 Hz) associated with an amplifier (Plug and Play 12 W) connected to a computer. During recordings in the tanks, the water pumps were switched off to reduce the background noise to a minimum. No animals were present in the tanks during these measurements. Sound was emitted for 2.5 s, with an intensity spread equally over a wide band of frequencies (between 0 Hz and 24 kHz) to simulate white noise. The sound was emitted 5 times at different distances from the hydrophone, from 0.1 to 1.5 m. Where peak frequencies appeared in the recorded white noise and had the same power spectrum level at different distances in a particular tank, these corresponded to the tank's resonant frequencies

(Akamatsu et al. 2002). Recorded peaks were then compared to the theoretical resonant frequencies ($f_{\text{rectangular}}$, Hz) of a rectangular glass tank with the dimensions L , W and H (after Akamatsu et al. 2002):

$$f_{\text{rectangular}} = \frac{c}{2} \sqrt{\left(\frac{l}{L}\right)^2 + \left(\frac{m}{W}\right)^2 + \left(\frac{n}{H}\right)^2} \quad (2)$$

where c is the sound velocity in the seawater (approximated at 1500 m s^{-1} in our case), l , m and n represent integers (≥ 1), and the combination of these is called the 'mode number'. The minimum resonant frequency is then defined at mode (1, 1, 1) for a particular tank dimension (Akamatsu et al. 2002).

Experiments

Sounds produced by individual lobsters during 2 different behaviours—feeding and response to stress—were recorded between March and May 2017 in the 8 tanks described above. During this period, 3 male lobsters molted within 1 wk of each other. We took advantage of this to compare sounds emitted between intermolt (i.e. hard shell) and postmolt (i.e. soft shell) lobsters under the same conditions.

Feeding

Rattles were emitted when lobsters were feeding. Six lobsters (3 postmolt males, 3 intermolt males) were used in each tank type, and sound recordings were made with all 3 different food types: frozen squid, frozen mackerel and fresh mussels. Sound recordings started at least 10 min after the introduction of the hydrophone in the tanks, and food was introduced into the tank below the hydrophone. Recordings ended when all added food had been consumed; the sessions lasted from 10 min to several hours.

Response to stress

Buzzing sounds were provoked by handling organisms as described in the literature for American lobsters (Fish 1966, Henninger & Watson 2005). All 17 lobsters were tested in each tank type. Individuals were gently lifted and maintained above the bottom of the tank for 20 s to 1 min in front of the hydrophone at distances between 10 and 20 cm for each recording.

Conditions of tank recordings

The hydrophone was suspended at the center of each tank, 20 cm above the bottom. Silicone mats (0.5 cm thick) were placed on the bottom of the glass-sided tanks to prevent sounds caused by the hard body parts of lobsters striking, or moving across, the glass. The 'daylight' conditions for experimental tanks were simulated using fluorescent light tubes above the tanks. The top of each tank was partially covered (25% of the total surface) by a polystyrene sheet above, but not touching the water surface, to create a shaded zone.

During sound recordings, the water pumps were switched off to reduce the background noise to a minimum. The background noise in the experimental tanks was recorded prior to each recording experiment without lobsters. Spectra for the background noise were flat (around 40 dB re $1 \mu\text{Pa}^2 \text{ Hz}^{-1}$), between 50 Hz and 75 kHz, with no typical peak frequencies compared to those that were present during experimental recordings with lobsters. At frequencies below 50 Hz, peaks were present (up to 90 dB re $1 \mu\text{Pa}^2 \text{ Hz}^{-1}$) and corresponded to instrumental self-noise from the recorder. These were excluded from the biological sounds analysis.

Then, an individual lobster was carefully transferred to the experimental tank from the separate holding tank. Acclimatization lasted at least 2 d before recordings began, and animals continued to be fed *ad libitum* during this period. Recording sessions started after the individuals were considered acclimatized to the presence of the hydrophone in their tanks (i.e. when attacks on the hydrophone stopped). After recordings, individuals were returned to the separate holding tank, and sound files were archived for analysis.

Sound analyses

Acoustic characteristics of recorded sounds

Recordings of raw sounds (files in .wav format) and videos were analyzed simultaneously to associate particular sounds with behavioural events. Based on this file annotation, each sound type from the different recordings was extracted manually using Audacity® (Version 2.1.1; Audacity Team 2015). Then, sub-sampled data from the converted recordings were analyzed between 1 and 78 kHz for the identified rattle sequences and between 60 and 500 Hz for identified buzzing sound sequences. All sequences were

processed using custom-made MATLAB (Version 9.1; 2016b) scripts. The following characteristics were calculated.

The sound pressure level (SPL, in dB re 1 μPa) was calculated for a time window equal to the length (T) of the selected sound (Erbe 2010). For characterizing broadband and transient rattles, the peak-to-peak SPL, SPL_{pp} , was calculated as:

$$\text{SPL}_{\text{pp}} = 20\log[\max(p(t)) - \min(p(t))] \quad (3)$$

where $\max(p(t))$ is the maximum value and $\min(p(t))$ the minimum value for the period, T . For continuous and narrowband buzzing sounds, the root-mean-square SPL, SPL_{rms} , was calculated as:

$$\text{SPL}_{\text{rms}} = 20\log\left(\sqrt{\frac{1}{T} \int_T p(t)^2 dt}\right) \quad (4)$$

The power spectral density was estimated by the periodogram, γ (in dB re 1 $\mu\text{Pa}^2 \text{Hz}^{-1}$):

$$\gamma(f) = |P(f)|^2 \quad (5)$$

with $P(f)$ being the Fourier transformation at frequency f of the time-domain signal $p(t)$:

$$P(f) = \int_T p(t)e^{-2j\pi ft} dt \quad (6)$$

The peak frequency (f_p , in Hz) is defined as the frequency at which the power spectral density is maximal. If the power spectral density contains several peaks, the frequency of the largest peak is called the first peak frequency.

Frequency bandwidth (B , Hz), was estimated as the measurement of the spread of the power spectral density (standard error) around the first peak frequency:

$$B = \frac{\sqrt{\int (f - f_p)^2 \gamma(f) df}}{\int \gamma(f) df} \quad (7)$$

Finally, in addition to rattles composed of trains of pulses (Fig. 1), we also calculated: (a) the duration of the entire sound (T_1 , in ms); (b) the pulse-to-pulse time interval (T_2 , in ms); (c) the total number of pulses per train (n); and (d) the pulse rate (R , in Hz), defined as the number of pulses per train (n) divided by the sound duration (T_1). Time characteristics were calculated using the first attack of each pulse (Fig. 1).

Statistical analysis

Characteristics of postmolt and intermolt lobsters' buzzing sounds were tested to determine whether differences between groups were significant. Considering the small number of samples, and assuming that calculated variables for each individual can be assimilated to a random distribution, the non-parametric Mann-Whitney (MW) test was used to determine whether their probability distributions were identical (significance level, $\alpha = 0.05$).

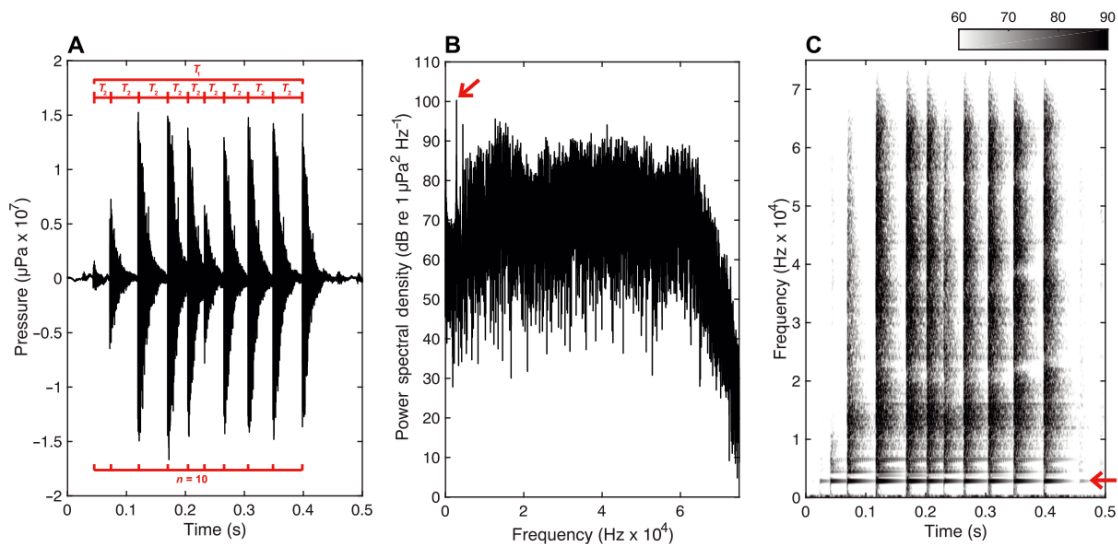


Fig. 1. Example of a typical rattle emitted in a 0.105 m^3 glass tank by a European lobster (10.6 cm carapace length) plotted using 3 different time-series analyses. (A) Oscillogram, showing how the following characteristics were measured: the number of pulses per train (n), the duration of the entire sound (T_1) and the pulse-to-pulse time interval (T_2). Time characteristics were calculated using the first attack of each pulse. (B) Acoustic spectrum (FFT size: 78126). (C) Spectrogram (FFT size: 1024; Hamming window: 501 points; 99% overlap). The red arrow indicates the first peak frequency (2.8 kHz) and corresponded to the minimum resonant frequency computed for the 0.105 m^3 tank (2.9 kHz). The color scale is in dB re 1 $\mu\text{Pa}^2 \text{Hz}^{-1}$

RESULTS

Distortion of a known sound through tank reverberation

From a white noise emitted, i.e. with the power spectral density being constant over a wide band of frequencies, signals recorded in different types of tanks were not flat and showed several peaks (Table 1). The first peak frequencies decreased as the dimensions of the tanks increased, ranging from 2.8 kHz for a 0.105 m³ tank to 1.8 kHz for the 2.34 m³ tank. These peaks occurred at frequency values matching the minimum theoretical resonant frequency calculated in the 0.105 m³ tank at 10 and 32 cm from the hydrophone (2.8 and 2.9 kHz, respectively). The same result was found for the 0.47 m³ tank at 10 cm from the hydrophone (2 kHz), while peaks at 2.7 kHz at 42 and 72 cm were also found, corresponding to another resonant frequency calculated with the mode (2, 2, 1), even if the peak at 2 kHz was still present. For the 2.34 m³ tank, the first frequency peak was 1.8 kHz for the 3 distances from the hydrophone, and corresponded to a resonant frequency of mode (1, 1, 2); a peak at 1.5 kHz (the minimum resonant frequency of this tank) was also present. The power spectral density of the recorded white noise showed several other peak frequencies up to the minimum resonant frequency in each tank (until almost 20 kHz).

Fig. 1 shows a rattle emitted by an intermolt male lobster (10.6 cm in CL) during feeding experiments in a 0.105 m³ tank. The first peak frequency of this broadband sound was 2.8 kHz (red arrow in Fig. 1), as also found for the recorded white noise in the same tank. It corresponded to the minimum resonant frequency of this tank, showing a net distortion of the sound.

Taking into account these results, we therefore calculated only time characteristics for the rattles, and SPL and spectral characteristics for buzzing sounds.

Recordings made during feeding

Rattles were defined as sound bursts consisting of a pulse train over a broadband spectrum (Fig. 1). A total of 168 rattles were recorded during feeding experiments with the 6 male lobsters (Table 2). These occurred when animals were feeding on all 3 types of foods. For almost 1 mo after molting, we did not record any rattles from the 3 soft lobsters, regardless of food type consumed. The time characteristics of these sounds were highly variable (Table 2): T_1 varied between 44 and 960 ms (mean \pm SD, 223.6 \pm 145.2 ms), T_2 varied between 1 and 89 ms (65.1 \pm 13.8 ms), n varied between 3 and 41 pulses per train (12 \pm 7.8) and R varied between 6.11 and 200 Hz (65.1 \pm 39.2 Hz).

Table 1. Sound characteristics calculated for artificial white noise emitted at different distances from the source and in different tanks used for sound recording experiments. Results are presented as mean (\pm SD). No animals were present during these recordings. f_p : peak frequency; $f_{\text{rectangular}}$: theoretical resonant frequency

Tank size (m ³)	— 0.105 —		— 0.47 —			— 2.34 —		
Distance from hydrophone (cm)	10	32	10	42	72	10	80	150
f_p (kHz)	2.8 (0)	2.8 (0)	2 (0)	2.7 (0)	2.7 (0)	1.8 (0)	1.8 (0)	1.8 (0)
$f_{\text{rectangular}}$ (kHz)	2.9	2.9	2	2	2	1.5	1.5	1.5

Table 2. Sound characteristics calculated in the European lobster rattles and buzzing sounds. The results are presented as means (\pm SE). I: interference due to tank reverberation; NA: not applicable. T_1 : duration of the entire sound; T_2 : pulse-to-pulse time interval; n : total number of pulses per train; R : pulse rate; SPL_{pp}: peak-to-peak sound pressure level; SPL_{rms}: root-mean-square sound pressure level

	No. of ind.	T_1 (ms)	T_2 (ms)	n	R (Hz)	SPL (dB re 1 μ Pa)	First peak frequency	Second peak frequency	Band width
Rattles	6	223.6 (145.2)	12.4 (13.8)	12 (7.8)	65.1 (39.2)	SPL _{pp} I	kHz I	kHz I	kHz I
Buzzing sounds	7	230 (187.5)	NA	NA	NA	SPL _{rms} 106.4 (6.1)	Hz 100.9 (19.6)	Hz 201.8 (39.2)	Hz 56.1 (22.5)

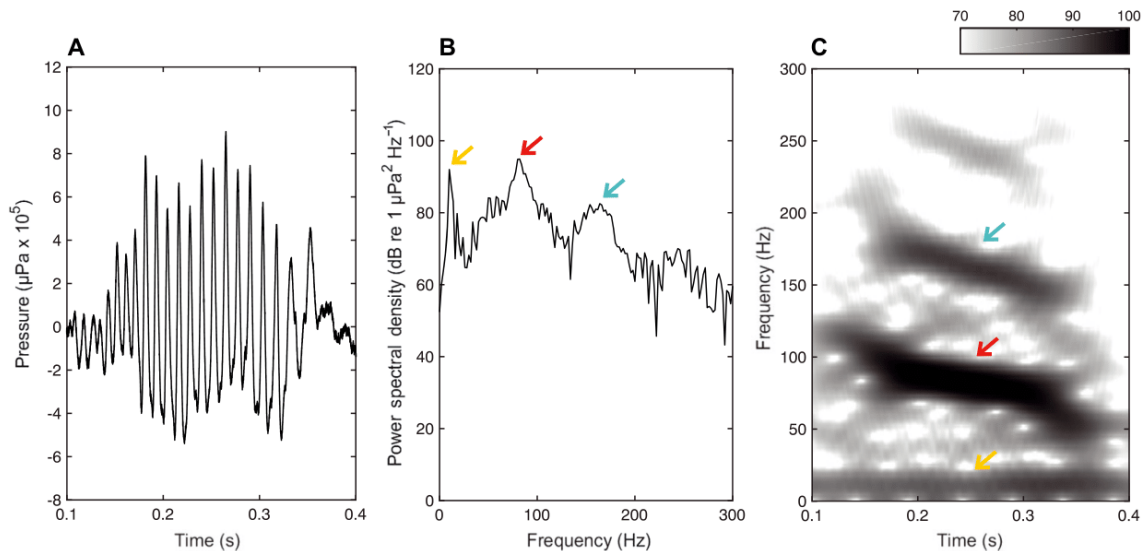


Fig. 2. Example of a buzzing sound produced in a 0.105 m^3 glass tank by a 'soft' (postmolt) European lobster (9.5 cm carapace length) plotted using 3 different time-series analyses. (A) Oscillogram; (B) acoustic spectrum (FFT size: 46876); (C) spectrogram (FFT size: 2048; Hamming window: 501 points; 99% overlap). The yellow arrow indicates instrumental self-noise from the recorder, the red arrow indicates first peak frequency and the blue arrow indicates the second harmonic. The color scale is in $\text{dB re } 1 \mu\text{Pa}^2 \text{ Hz}^{-1}$

Carapace vibration in a response to stress

Buzzing sounds were defined as continuous sounds with a narrowband spectrum. A total of 189 buzzing sounds were recorded as a stress response by 7 individuals (1 intermolt female, 3 intermolt males and 3 postmolt males, ranging from 9 to 12 cm in CL) of the 17 lobsters tested (Table 2). These sounds occurred in association with carapace vibration (felt by hand) and were often associated with tail flips. Buzzing sounds were also recorded from the 2 most aggressive intermolt male lobsters (10.6 and 12 cm in CL) just before they attacked the hydrophone in the 0.105 m^3 tanks during feeding experiments. These sounds had a mean first peak frequency of 100.9 Hz (range 66.6 to 152.6 Hz) and were mainly (92% of the analyzed recordings) accompanied by a second, strong harmonic with a mean frequency at second peak intensity of 201.8 Hz (range 123.2 to 305.2 Hz). The number of harmonics detected varied from 2 to >10 for the most intense buzzing sounds. The buzzing sounds tended to decrease in frequency over time (Fig. 2), showing a net, mean frequency modulation of 56.1 Hz when the onset dominant frequency was compared to the dominant frequency at the end of the sound. The harmonics showed the same pattern. Overall, the buzzing sounds had a mean SPL of 106.4 $\text{dB re } 1 \mu\text{Pa rms}$ (range 92.1–119.3 $\text{dB re } 1 \text{ Pa rms}$), and could be as long as 1600 ms (mean = $230 \pm 187.5 \text{ ms}$; Table 2).

The 3 soft lobsters tested during the feeding experiments were also observed to be capable of emitting buzzing sounds, and more frequently than the intermolt lobsters. We observed no significant differences in buzzing sounds emitted between postmolt and intermolt lobsters in the sound duration, SPL and peak frequencies (MW, $p > 0.05$). However, the bandwidth was significantly larger for intermolt lobsters compared to postmolt lobsters (MW, $p < 0.05$). In addition, one postmolt lobster (12 cm CL) emitted 3 buzzing sounds with the highest SPL (>119 $\text{dB re } 1 \mu\text{Pa rms}$) values observed here.

DISCUSSION

Quantification of bioacoustic signatures in tanks

Characterizing reverberation in the different tanks required using known sound in order to be able to quantify how the tank geometry distorted sounds. As expected, reverberation distorted the white noise used in our tests for all the tanks. Several peak frequencies appeared corresponding to the calculated theoretical resonant frequencies of the different tanks (Table 1). The same result was also found for recordings of broadband rattles emitted by the lobsters in these tanks (Fig. 1). Based on these observations, and inspired by Akamatsu et al. (2002), we summarized these results as a guideline for charac-

terizing marine crustaceans' sounds in small tanks (Table 3).

Although we recognize that this information exists in various forms in the acoustic literature, it seems important to present these guidelines directly to the bioacoustic community, where studies of crustaceans in tanks are frequently described in the literature (Hazlett & Winn 1962a,b, Meyer-Rochow & Penrose 1974, 1976, Mulligan & Fischer 1977, Patek & Caldwell 2006, Patek & Baio 2007, Patek et al. 2009, Buscaino et al. 2011, 2015, Coquereau et al. 2016a,b). Most of these studies did not attempt to quantify reverberation effects on the broadband and transient sounds. For example, Meyer-Rochow & Penrose (1976) found that spectral characteristics of the squeak produced by the rock lobster *Palinurus longipes* were strongly influenced by the size and material of their recording tanks, and finally concluded that the bandwidth was uniform over the audio range. Different types of sound characteristics are commonly presented in the bioacoustic literature, including: spectral characteristics (peak frequencies, bandwidth), time (duration, pulse rate, time inter-pulse) and measured energy (SPL) and source energy ((SL; i.e. SPL estimated at one distance from a source)). In our study, we have shown that spectral characteristics in transient and broadband sounds (such as rattles), which are usually produced by crustaceans, cannot be calculated in tanks.

In contrast, low-frequency sounds (such as buzzing) can be measured in tanks. Yet other analytical methods based on SPL, such as SL estimated at 1 m from a source, do not appear reliable because of the sound

Table 3. Acoustic methodology showing which sound characteristics (spectral, time, energy) can be calculated in small tanks (example: 0.105 m³) depending on the type of recorded sound. The sound characteristics were calculated selecting the entire sound. f_{res} : minimum resonant frequency of the tank; SPL: sound pressure level; SL: source energy (i.e. SPL estimated at one distance from a source)

Sound characteristic	Sound frequency	
	$<f_{res}$	$>f_{res}$
Spectral shape	Ok	No
Time (duration)	Ok	No
Time (beginning of the sound)	Ok	Ok (if sound is separated enough from other sound)
SPL	Ok	Possible if $f \gg f_{res}$
SL	No	Possible if $f \gg f_{res}$
Examples from this study	Buzzing (narrowband and continuous sounds)	Rattles (broadband and transient sounds)

propagation models in use. As a reminder, Rogers et al. (2016) investigated transmission loss in the same range as our buzzing sounds in very small (0.03 m³) tanks. They showed an exponential decrease of 35 dB between 10 and 30 cm from the hydrophone, whereas based on the spherical spreading propagation model commonly used, the expected transmission loss would be estimated at only 10 dB. These results suggest that the SL values would also be unreliable in our experiments (Table 3). One solution to infer crustacean SLs in tank experiments would be to develop new propagation models, such as the one proposed by Rogers et al. (2016). This would require very accurate estimations of the source position, which is unfortunately not possible at this time for crustaceans because they are allowed to move freely in the tanks and thus cannot be considered as a fixed-point source.

Comparison with the bioacoustic literature

Rattles emitted by *Homarus gammarus* during feeding were defined as trains of broadband pulses. In the present study, first peak frequency, bandwidth and SPL were not assessed because the recordings were highly distorted due to reverberation (Table 1, Fig. 1). Other authors have suggested that broadband and transient sounds emitted by marine arthropods can be characterized by sampling only the initial part of the signal (e.g. an interval less than 0.2 ms), which would correspond to a 1-way emission just before reverberation starts (Coquereau et al. 2016a). This method was not applied in the present study, because it would not determine whether the frequencies of transient sounds vary over time. For example, beaked whales emit sounds with very fast frequency modulations (Baumann-Pickering et al. 2013), which this type of signal sub-sampling would not account for. In addition, calculating a power spectral density with too few points may not fulfill the conditions for accurately estimating spectral characteristics. Thus, we preferred to focus on descriptors not impacted by tank reverberation, such as time characteristics: the duration of the entire sound, pulse-to-pulse time interval, number of pulses per train, and pulse rate.

The time characteristics of the lobsters' rattles were consistent with the feeding sounds previously reported for tropical spiny lobsters. The mean entire duration of 223 ms was of the same order of magnitude (Moulton 1957: 250 ms for *Palinurus argus*; Meyer-Rochow & Penrose 1976: 153 ms for *P. longipes*). Other types of feeding sounds recorded in

temperate crustaceans had longer durations (Coquereau et al. 2016a: approximately 600 ms for *Maja brachydactyla*; Coquereau et al. 2016b: approximately 400 ms for *Cancer pagurus*). The number of pulses per signal was calculated as (mean \pm SD) 12 ± 7.8 on average for lobster rattles, which compares favourably with the reported 5 to 10 pulses per signal for spiny lobsters (Moulton 1957 for *P. argus*; Meyer-Rochow & Penrose 1976 for *P. longipes*), but is smaller than the mean of 29 ± 11 pulses per signal reported for *M. brachydactyla* (Coquereau et al. 2016a). In a general way, time characteristics in lobsters' rattles were highly variable. These rattles may be produced by the friction between mouthparts (called 'mandible grinding' in Meyer-Rochow & Penrose 1976), suggesting unintentional sounds are produced and may thus account for some of this reported variability.

Similar to the American lobster, the European lobster also produces a buzzing sound (Henninger & Watson 2005) that could be recorded by the hydrophone (Fig. 2). First peak frequencies in the European lobster were within the range of reported first peak frequencies by Fish (1966; 100 to 130 Hz) and Henninger & Watson (2005; 87 to 261 Hz) for the American lobster. Sound durations, however, were highly variable (50 to 1600 ms), but were in the same range as those reported by both Fish (1966; between 100 and 500 ms) and Henninger & Watson (2005; between 68 to 1720 ms). In addition, the mean SPL was estimated to be about 10 dB re 1 μ Pa rms lower than for American lobsters (between 116.5 and 118.5 dB re 1 μ Pa rms; Fish 1966, Henninger & Watson 2005, Ward et al. 2011). A second strong harmonic was also observed in the European lobster that has not been described for the American lobster. The bandwidth showed how the buzzing sound decreased in frequency over time, suggesting that these types of modulations may be a characteristic of this sound. Similar results have indeed already been reported from stomatopods or mantis shrimp *Hemiquilla californiensis*, which generate tonal, low frequency sounds called 'rumbles' (Patek & Caldwell 2006). These same authors also observed that the first peak frequencies of the rumbles were accompanied with a second strong harmonic. Staaterman et al. (2011) reported 'rumbles' recorded in the field close to our results for lobsters buzzing sounds (an average dominant frequency of 167 Hz and a mean duration of 200 ms). Apart from these 3 marine crustacean species, a number of other marine organisms also generate similar low frequency sounds, including whales and fish (Clark & Johnson 1984, Connaughton 2004, Maruska & Mensinger 2009).

Is there a role for buzzing sounds emitted by the European lobster?

Because of the lack of field observations in the ecological context in which buzzing sounds are generated, it is only possible to speculate about their role based on comparisons with other organisms. As lobsters produced vibrations when handled (this was already demonstrated in Henninger & Watson 2005), it has been suggested that buzzing sounds may serve to deter potential predators. These sounds have a narrow bandwidth, suggesting that only organisms capable of perceiving sounds in the same band of frequencies might be targeted. Ward et al. (2011) showed that American lobsters vibrated when approached by 2 species of fish in a circular tank (cod and striped bass). Interestingly, the sound sensitivity of cephalopods, including octopuses, seems to be limited to low frequencies (100–200 Hz; Williamson 1988, Packard et al. 1990, Mooney et al. 2010). As the octopus is a well-known predator of European lobsters (Barshaw et al. 2003), these buzzing sounds may indeed be meant as a deterrence.

Buzzing sounds were also recorded just before 2 male lobsters attacked the hydrophone during feeding experiments. Lobsters are known to be territorial species living in burrows similar to those of mantis shrimp (Dingle & Caldwell 1969), and buzzing sounds might help to send signals of their presence to conspecifics in addition to chemical cues (Skog et al. 2009), to maintain territory. This type of behaviour was previously described in mantis shrimp (Patek & Caldwell 2006). Staaterman et al. (2011) recorded multiple rumbles of mantis shrimp in the field, which they termed 'chorusing'. These rhythmic series, called 'rumble groups', may even constitute a type of conspecific communication.

Crustaceans, including lobsters, lack gas-filled organs (i.e. swim bladders) required for pressure detection, but may be still capable of detecting low-frequency acoustic stimuli arising from particle motion (Popper et al. 2001, Edmonds et al. 2016, Popper & Hawkins 2018), such as the buzzing sounds. A large diversity of sensory receptors has been described in crustaceans, including statocysts and sensory hairs (Popper et al. 2001, Radford et al. 2016). Two types of putative acoustic receptors on *H. gammarus*—hairfan and hair-peg organs—have been implicated in low-frequency sound and water-current detection (Laverack 1962, 1963). In addition, one study focused on sound detection by *H. americanus* showed that they are capable of detecting sounds, with the most sensitive responses at the lower frequencies (be-

tween 18.7 and 150 Hz; Offutt 1970). Our results also showed that 'soft' (postmolt) lobsters were able to produce buzzing sounds with the same characteristics (except for their bandwidth) as 'hard' (intermolt) lobsters. This suggests that the buzzing sounds may be a means of communication. New studies are needed to clearly examine the physiological and behavioural responses of European lobsters to such sounds.

Henninger & Watson (2005) showed that only 7.5% of their American lobsters (from a total of 1723 individuals tested) vibrated when handled, despite the fact that all lobsters have the anatomical capacity to produce these sounds. Their year-long survey demonstrated that all size classes of American lobster can produce buzzing sounds, with a similar distribution for both males and females. In the present study, we observed that only 7 out of the 17 lobsters tested vibrated and produced buzzing sounds when handled. We recorded buzzing sounds in 6 male and only 1 female lobster, meaning that 1 male and 9 female lobsters did not vibrate. Due to the low number of individuals tested, and to the stress of captivity, which could habituate lobsters to being disturbed, we cannot yet explain this difference in sound production between individuals. Further, we do not know at which stage of their life cycle the mechanism of sound production becomes operational and biologically useful in these animals.

Conclusions

Passive acoustic studies of marine crustaceans would clearly benefit from field measurements. Indeed, we emphasize that field studies are required to confirm the acoustic findings presented in this study and to test hypotheses about sound transmission and detection in natural soundscape. Preliminary characterization of European lobsters' buzzing sounds, however, suggests these could be difficult to record because they may be masked by other sources of low-frequency sounds, such as sea surface agitation related to wind speed (Wenz 1962) and anthropogenic noise (Clark et al. 2009). In conclusion, acoustic measurements in carefully controlled laboratory conditions together with behavioural observations remain an essential first step, and they should serve as a basis of comparison for any subsequent *in situ* research and monitoring projects.

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RESEARCH ARTICLE

Acoustic behaviour of male European lobsters (*Homarus gammarus*) during agonistic encounters

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ABSTRACT

Previous studies have demonstrated that male European lobsters (*Homarus gammarus*) use chemical and visual signals as a means of intraspecific communication during agonistic encounters. In this study, we show that they also produce buzzing sounds during these encounters. This result was missed in earlier studies because low-frequency buzzing sounds are highly attenuated in tanks, and are thus difficult to detect with hydrophones. To address this issue, we designed a behavioural tank experiment using hydrophones, with accelerometers placed on the lobsters to directly detect their carapace vibrations (i.e. the sources of the buzzing sounds). While we found that both dominant and submissive individuals produced carapace vibrations during every agonistic encounter, very few of the associated buzzing sounds (15%) were recorded by the hydrophones. This difference is explained by their high attenuation in tanks. We then used the method of algorithmic complexity to analyse the carapace vibration sequences as call-and-response signals between dominant and submissive individuals. Even though some intriguing patterns appeared for closely size-matched pairs (<5 mm carapace length difference), the results of the analysis did not permit us to infer that the processes underlying these sequences could be differentiated from random ones. Thus, such results prevented any conclusions about acoustic communication. This concurs with both the high attenuation of the buzzing sounds during the experiments and the poor understanding of acoustic perception by lobsters. New approaches that circumvent tank acoustic issues are now required to validate the existence of acoustic communication in lobsters.

KEY WORDS: Passive acoustics, Accelerometer, Buzzing sound, Carapace vibration, Tank, Acoustic communication, Dominance, Sound attenuation

INTRODUCTION

Sounds can be used by marine organisms to convey information. Numerous studies have demonstrated that marine mammals and fish use sounds to navigate, find food, communicate with conspecifics or even deter predators (e.g. Tyack and Clark, 2000; Ladich, 2015). By comparison, the potential role(s) of sounds amongst marine invertebrates is poorly described (Taylor and Patek, 2010; Edmonds et al., 2016).

For instance, only a few crustacean species have been shown to produce sounds during behavioural interactions. The tropical spiny lobster (*Panulirus argus*) produces antennal rasps when attacked by predators (Bouwma and Herrnkind, 2009). Mantis shrimp (*Hemisquilla californiensis*) rumble to maintain their territories against conspecifics (Patek and Caldwell, 2006; Staaterman et al., 2011). Semi-terrestrial crabs (the Ocypodidae) are known to produce stridulations that attract females to their burrows for mating (Popper et al., 2001). Other crustacean species have also been shown to produce sounds, but the lack of relevant behavioural studies does not yet permit validation of potential ecological roles for these sounds. In the temperate coastal waters of Brittany (France), several crustacean species produce a large diversity of sounds, but their ecological roles, if any, are unknown (Jézéquel et al., 2018, 2019).

Lobsters, particularly the American lobster (*Homarus americanus*), have been identified as a good study model for analysing complex behaviours (Scrivener, 1971; Atema and Voigt, 1995; Huber and Kravitz, 1995). Male *H. americanus* lobsters exhibit highly aggressive behaviours towards each other. Indeed, they use agonistic encounters to establish and maintain their dominance within a group to gain better access to shelters and females for reproduction (Scrivener, 1971; Atema and Cobb, 1980; Atema and Voigt, 1995). When two individuals meet, they exhibit an array of agonistic behaviours ranging from visual displays to physical contact (Scrivener, 1971; Huber and Kravitz, 1995; Breithaupt and Atema, 2000). The main factor influencing the outcome of an agonistic encounter is body size: larger individuals have a greater chance of winning an encounter (Scrivener, 1971). This results in shorter behavioural sequences compared with those for size-matched lobsters where their aggressive behaviours lead to highly stressful conditions (Atema and Voigt, 1995). The loser of an encounter avoids the winner afterwards, and dominance is maintained through a variety of signals. Chemical signals (i.e. pheromones) released in urine appear to be the main means of preserving the memory of the outcome between pairs of individuals, post-encounter (Breithaupt and Atema, 1993; Karavanich and Atema, 1998; Breithaupt et al., 1999). Recently, Gherardi et al. (2010) and Bruce et al. (2018) showed that visual recognition of specific individuals also plays a role. The ability to recall the outcome of past encounters may help individual lobsters to avoid additional fights and lower their future risk of injury (Breithaupt and Atema, 2000).

A recent study has shown that, similar to *H. americanus*, the European lobster (*Homarus gammarus*) also emits buzzing sounds when stressed (Jézéquel et al., 2018). These sounds are produced through the rapid contraction of internal muscles located at the base of their second antennae, which causes the carapace to vibrate (Mendelson, 1969). These ‘buzzing’ sounds are characterized by low frequencies (~100 Hz) and have a relatively long duration (~200 ms; Henninger and Watson, 2005; Jézéquel et al., 2018).

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Ward et al. (2011) suggested that *H. americanus* may only use these sounds to deter predators. Interestingly, earlier studies indicated that few buzzing sounds were produced during agonistic encounters in male *H. americanus* and it was then concluded that these sounds do not have a role for intraspecific interactions (Scrivener, 1971; Atema and Voigt, 1995; Atema and Cobb, 1980). Hence, no study has examined the ecological role of these buzzing sounds and only one has described the behavioural patterns in *H. gammarus* during agonistic encounters (Skog et al., 2009).

The primary aim of the present study was to: (1) test whether male *H. gammarus* emit buzzing sounds during agonistic encounters, and (2) test the potential role of these sounds as acoustic communication between lobsters. For this purpose, we designed an experimental laboratory set-up whose main feature was a tank containing the lobsters under study. The set-up also combined hydrophones to record their buzzing sounds in the tank, accelerometers on the lobsters to record their carapace vibrations (i.e. the source of the buzzing sounds) and cameras to record animal behaviour. Firstly, we developed a detailed ethogram based on the video recordings of the encounters. Secondly, we analysed the behavioural sequences between dominant and submissive individuals. Lastly, we examined whether the sequences of buzzing sounds produced by two individuals depended on their relative size differences. We then analysed these as call-and-response signals to explore their potential role for the communication of dominance.

MATERIALS AND METHODS

All laboratory experiments were carried out at the research facilities of the Océanopolis public aquarium located in Brest (France).

Animal collection, characteristics and care

For these experiments, a total of 24 *H. gammarus* (Linnaeus 1758) male individuals, with carapace length (CL; measured from the eye socket to the posterior carapace margin for lobsters) between 8.7 and 13 cm, were used. They were collected carefully by hand while snorkelling in the Bay of Plougonvelin (Brittany, France) at water depths of between 1 and 10 m. Two samplings were done in May and July 2018; 12 individuals were collected during each session. Only intermoult individuals (following the description in Aiken, 1973) with full sets of undamaged appendages were collected and used for this study.

After capture, lobsters were separated randomly into two groups of 6 individuals each, and then transferred to different holding tanks. One group was held in a large shaded, polyester circular tank (radius 4 m, effective height 1.13 m, seawater volume 14.2 m³). The second group was held in two identical plastic rectangular tanks (1.50 m×1.00 m×0.5 m length×width×effective height; seawater volume 0.75 m³) with 3 individuals per tank, separated by plastic

dividers. In the communal tanks, the lobsters' claws were bound with numbered rubber bands to avoid injury. These also identified each individual lobster. All holding tanks were continuously supplied with sand-filtered, UV-sterilized seawater pumped from the Bay of Brest. Temperature, salinity and animal condition were controlled twice a day. During holding, temperature varied between (mean±s.d.) 14.8±1°C (in May and June) and 17.5±0.5°C (in July and August) and salinity between 34.4±0.3 and 34.9±0.1. Animals were fed with fresh pieces of fish (mackerel) and cephalopod (squid) *ad libitum*. They were kept under the natural photoperiod in the large circular tank, and under a 12 h:12 h photoperiod in the smaller tanks, the daylight condition being simulated by fluorescent light tubes above the tanks. Sections of rigid PVC drainage pipes were provided in abundance as shelters. Animals were acclimatized for at least 1 month in these conditions before they were used in the experiments.

Experimental set-up

All experiments were done in a dedicated plastic tank (1.13 m×0.73 m×0.5 m; 0.4 m³) placed in a quiet room, isolated from the main activities of the aquarium facilities (Fig. 1). The bottom was covered with a thin layer of sand, 5 cm deep, to provide a foothold for the animals. Two LED light strips (B0187LXUS2, colour temperature 4500 K) were placed 50 cm above the tank to ensure good visibility for video recording by the cameras. The experimental tank was divided into two equal volumes by a removable, opaque, Plexiglas divider (6 mm thick) installed in the middle of the tank prior to introducing the animals (Scrivener, 1971; Huber and Kravitz, 1995; Skog et al., 2009). To do this, plastic gutters were epoxy glued on the vertical sides and along the bottom of the tank. This permitted the divider to easily slide up at the start of each experiment. The edges of these gutters were silicone sealed to eliminate any water exchange while the divider was in place. The barrier prevented the exchange of chemosensory and visual cues between the two lobster opponents before the agonistic encounter was started by removal of the divider.

Data recording

Buzzing sounds: hydrophones

Sounds were recorded using two pre-amplified hydrophones (HTI-92-WB, High Tech Inc., Long Beach, MS, USA), with a sensitivity of −155 dB re. 1 V μPa^{−1} and a flat response between 2 Hz and 50 kHz. Hydrophones were connected to a compact autonomous recorder (EA-SDA14, RTSys, Caudan, France) with a gain of 14.7 dB, and were powered by battery to limit electronic self-noise. Recordings were made with a sampling frequency of 156 kHz at 32-bit resolution. Even though buzzing sounds are characterized by low frequencies (~100 Hz; Jézéquel et al., 2018), we chose a high

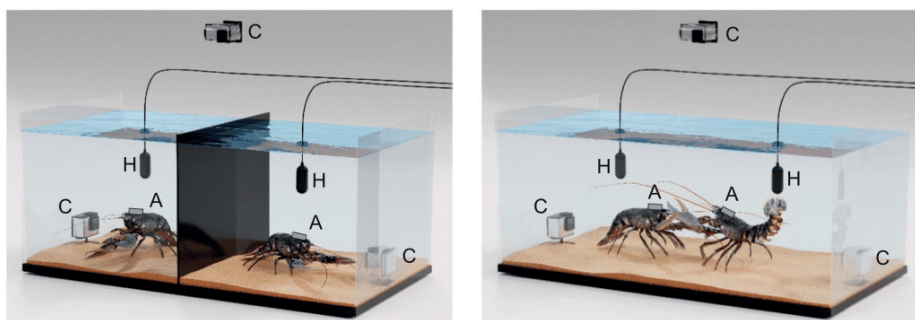


Fig. 1. Experimental set-up of the agonistic encounters performed with male European lobsters, *Homarus gammarus*. Individuals were first isolated for a period of 15 min on either side of the experimental tank (left), and then the divider was lifted and we recorded agonistic encounters for another 15 min. For these experiments, we used several recording devices: two hydrophones (H), two accelerometers (A) and three cameras (C).

sampling frequency because we wanted to cover a large frequency band, in case the lobsters produced new sounds during the experiments.

We used two hydrophones in the experimental tank to ensure most of the buzzing sounds emitted by individuals during the agonistic encounters could be recorded. One was placed in each compartment of the tank, 30 cm above the substrate, and they were separated by 55 cm from each other (Fig. 1). Based on our earlier work to determine the acclimation state of the animals used (Jézéquel et al., 2018), this installation did not perturb the individuals.

Carapace vibrations: accelerometers

During preliminary trials, we noted that very few buzzing sounds were recorded by the hydrophones during agonistic encounters. Lobsters emit buzzing sounds through rapid contractions of internal muscles located at the base of their antennae, which vibrate the carapace (Henninger and Watson, 2005); we therefore added accelerometers on their carapaces as a means to detect carapace vibration events, independently of the hydrophones.

One small AX-3 data logger (23×32.5×8.9 mm, mass 11 g; Axivity Ltd, Newcastle Helix, UK) was glued with 3 min underwater epoxy to the dorsal carapace of each lobster, near the eye sockets at the base of the second antennae (Fig. 1). The *x*-axis was oriented parallel to the longitudinal body axis, which is also parallel to the internal muscles responsible for the carapace vibration (Henninger and Watson, 2005). The accelerometers were set to record acceleration in all three axes (range ±16 g, 156.96 m s⁻²) with a sampling frequency of 3200 Hz and a 13-bit resolution. The accelerometers had a 512 MB memory card onboard. Each accelerometer was waterproofed before attachment by encasing it in a polyethylene film sealed shut with heat-shrink tape. Air trapped inside the polyethylene film made the accelerometer loggers neutrally buoyant in seawater. All accelerometers were marked with unique numbers to associate them with particular individuals. This technique permitted us to link each carapace vibration recorded to an individual and also to validate the buzzing sounds recorded with the hydrophone recordings. As stated above for the hydrophones, we did not observe any evidence that the presence of the sensors perturbed their movements during the experiments.

Movements: video

Visual observations and video recordings were made during all experiments using three GoPro® HERO3 cameras. Two cameras were placed in the bottom of the tank at either end against the walls, and a third camera was placed 50 cm above the water surface of the tank (Fig. 1). Videos used a recording rate of 29.97 frames s⁻¹ with an image resolution of 1920×1080 pixels.

Data synchronization

To ensure that all the data streams could be re-synchronized, we used a synchronization procedure at the end of the experiments. First, the accelerometers were gently taken off the lobsters and placed on the sand in the middle of the tank, and the two lobsters were returned to their holding tanks. Then, five sharp raps were made on the tank walls that could be used to synchronize all three types of recording device (hydrophones, accelerometers and GoPros).

Experimental design

Experiments were performed during June and August 2018 in the experimental tank described above. During each experiment,

seawater temperature was measured using a HOBO Pendant G data logger (UA-004-64, Onset Computer Corporation). Seawater temperature in the experimental tank was 17.11±0.14°C (mean±s.d.) in June and 18.44±0.12°C in August.

Agonistic encounters were set up between two categories of lobsters: size-matched male lobster pairs (difference in carapace length, $\Delta\text{CL}<5$ mm), and small and large male individuals ($\Delta\text{CL}>5$ mm). In fact, larger lobsters are more likely to win a fight if the ΔCL is more than 5 mm between the opponents, but at smaller size differences, the outcome is random (Scrivener, 1971). We formed pairs by taking one individual from each separately acclimated group to ensure that the individuals had no prior knowledge of each other (Karavanich and Atema, 1998). A total of 12 agonistic encounters (6 with $\Delta\text{CL}<5$ mm; 6 with $\Delta\text{CL}>5$ mm) were set up.

Because communal holding causes a general reduction of aggressiveness in lobsters (Breithaupt and Atema, 2000), we isolated the two selected individuals separately for 24 h in glass-sided rectangular tanks (0.60 m×0.50 m×0.35 m; 0.105 m³) after the accelerometers were attached. This allowed the lobsters to recover from handling. For this step, the bands on their claws were also released. Lobsters were not fed during this period.

The next day, these same individuals were placed in the prepared experimental tank, one on either side of the divider (Fig. 1). Experiments were performed between 16:00 h and 20:00 h. Recordings started when the individuals were placed in the tank. We recorded the first 15 min as control observations of the individuals while they were in isolation in their respective compartments. Next, we lifted the divider and continued recording the agonistic encounters that ensued for another 15 min. This corresponds to the expected minimum time for determining the outcome, according to Scrivener (1971). After the experiment, the accelerometers were removed from both animals, and the lobsters were returned to their holding tanks. Then, the data synchronization procedure (described in 'Data synchronization', above) was followed. Afterwards, the experimental tank was drained completely, thoroughly rinsed and refilled with fresh seawater, and the sand was replaced. Each individual was used only once during the study.

Data analysis

Sound data

Sound files (.wav) from the two hydrophones (30 min recordings each) were archived at the end of each experiment. They were first carefully visualized over the entire frequency band (between 0 and 78 kHz) by using the spectrogram mode in Audacity® (v2.1.1; www.audacityteam.org) to check for potential biological broadband sounds emitted by lobsters during experiments. Next, sound data were subsampled between 0 and 500 Hz and spectrograms were visualized a second time using custom-written MATLAB scripts (v9.1; The MathWorks, Natick, MA, USA). The characterization of buzzing sounds has been detailed in our earlier study (Jézéquel et al., 2018). As the aim of the experiments in the present study was to understand when these sounds were produced within the behavioural sequences, here we only report the basic descriptive statistics of the buzzing events recorded during the agonistic encounters.

Accelerometry data

Data from the accelerometers were downloaded using Open Movement GUI software (v1.0.0.37). Accelerometers record movements simultaneously on three axes as the relative change detected in gravitational acceleration, *g* (1 *g*=9.81 m s⁻²), and

carapace vibrations are known to have the same frequency as their associated buzzing sounds (~100 Hz; Henninger and Watson, 2005). After examination of the data on all three axes, we observed the strongest signals of the carapace vibrations were recorded on the *x*-axis, as expected. We thus used only the *x*-axis data to facilitate their detection among all the other high-amplitude signals related to the lobster movements (e.g. tail flips) by treating the data using a custom-written MATLAB script. We report here the number and timing of carapace vibration events recorded during each agonistic encounter for each individual. We also measured their duration (in ms) and peak frequency (in Hz).

Video annotation of movements during encounters

Video analysis consisted of annotating the visible movements performed by each individual during the encounters. Based on the extensive *H. americanus* literature (see Table S1) and preliminary tests, we built a description of movements (also termed ethogram) by annotating 30 associated movements for five different body parts (antennae, claws, legs, carapace, tail; Table 1). We focused on movements or actions initially instead of 'behaviours' because it allowed us to avoid subjective choices related to the sometimes ambiguous behaviours defined in the literature. Movement directions like 'walking away' and 'walking backward' were identified according to the direction of the body axis relative to the other individual. For example, 'walking away' for a lobster was defined as the direction of its rostrum that pointed away from its opponent, but does not necessarily mean it was escaping from its opponent. These 30 movements were annotated for each individual

and for all 12 agonistic encounters using the tools in BORIS (v6.3.9; Friard and Gamba, 2016).

Video data from each agonistic encounter comprised video recordings (30 min each) from each of the three cameras used in the experiments. We chose to annotate primarily videos from the plan view camera because this covered the entire experimental area and most of the movements were visible. We completed these observations by analysing the recordings from the two cameras placed in the bottom of the tank. This permitted us to visualize more precisely certain vertical movements made by the lobsters (e.g. high on legs, meral spread). All these annotations were then integrated with the annotation from the plan view camera for subsequent data treatment. Time energetic budgets were made for each movement and each individual (submissive and dominant) as percentages of the total length of the agonistic encounters (15 min).

Statistical analyses

Statistical analyses were performed using R v3.5.1 (<http://www.R-project.org/>). The mean percentage total time for each movement was tested for significant differences between dominant and submissive individuals in all 12 encounters. As these data were not distributed normally (Shapiro–Wilk test, $P < 0.05$), the non-parametric Mann–Whitney test (*U*-test) was used to determine whether their probability distributions were equal. The significance level for null hypothesis rejection was $\alpha < 0.05$. These results permitted us to associate sequences of movements typically shown by dominant and submissive individuals to particular behaviours based on the conventions used in the *H. americanus* literature (see Table S1).

Table 1. Ethogram of adult male European lobsters (*Homarus gammarus*) during agonistic encounters

Appendage	Movement	Description
Antennae	Antennae backward	Both antennae are positioned backward along the main body axis
	Antenna pointing	One, or both, antennae directed forward, toward opponent
	Antenna sensing	One, or both, antennae directed at opponent with lateral movements
	Antenna touching	One, or both, antennae continuously touching opponent
	Antennae up	Antennae pointing directly up, perpendicular to main body axis
	Antenna whipping	Lashing of opponent's body with antenna(e) in a sweeping motion
Claws	Claw boxing	One claw is pulled backward and then makes a quick 'hook' type strike directed toward the opponent's claw(s) or body
	Claws extended	Claws rest on substrate (in front of animal) and are stretched forward so that merus-carpus-propodus of both claws are aligned with body axis
	Claw forward	One claw is stretched forward (as a weapon) while the other is held close to the body (as a shield)
	Claw grasping	Clamping of claws onto opponent's claw(s) or body
	Claw locking	Crusher claws interlocked; resembles 'handshaking'
	Claws lunging	Thrusting claws forward
	Claw open	The dactyl of either, or both, claws fully open; generally crusher claw
	Claw pushing	Continuous pushing with claws on opponent's body
	Claw ripping	Rapid grasp and pull motion, with either claw, of opponent's claw(s) or body
	Claw scissoring	Both claws pulled backward and rapidly crossed in front of opponent's claw(s) or body in a scissor-like motion
	Claw snapping	Rapid opening and closing of seizer claw in direction of opponent
	Claws touching	Continuous touching of opponent with claws
	Meral spread	Both claws held wide apart above substrate facing opponent
	Legs	High on legs
Sand removing		Legs are used to remove sand, causing back and forth rocking movements of the body
Carapace	Facing	Body not moving and rostrum directed towards opponent
	Resting	Body not moving and rostrum not directed towards opponent
	Turning away	Body turns so rostrum points away from opponent
	Turning toward	Body turns so rostrum points toward opponent
	Walking away	Walking with rostrum pointing away from opponent
	Walking backward	Walking backward with rostrum directed toward opponent
	Walking parallel	Walking with rostrum parallel to opponent's body axis
Walking toward	Walking with rostrum pointing toward opponent	
Tail	Tail flipping	Rapid abdominal contractions which propel the lobster backward

The terms are described with respect to the direction of the movement performed by body appendages, and are not related to behaviours or ranks of aggression used in the *Homarus americanus* literature (see Table S1). The description was used to annotate movements from lobsters in videos of agonistic encounters.

Analysis of carapace vibration sequences

As our experiments were necessarily brief to avoid injury (15 min long; Scrivener, 1971) and each experiment was also unique, the carapace vibration sequences did not meet the criteria for classical statistical tests (Guarini et al., 2019). Because the development of a behavioural model was beyond the scope of the present work, we only considered whether the sequences of carapace vibrations recorded by the accelerometers on each individual during the agonistic encounters could not have been produced by a random process. Instead of classical tests, we used a definition of randomness for algorithmic complexity that was recently formalized for short series of fewer than 100 characters that are common in behavioural studies (Soler-Toscano et al., 2014; Zenil et al., 2015preprint; Gauvrit et al., 2016).

Algorithmic complexity offers an alternative means to evaluate the existence of ordered patterns in short sequences by assessing the computing effort needed to stimulate them (Zenil et al., 2018). The approach compares a given string with results from randomly selected Turing machines calculating the likelihood that the string could be reproduced by these algorithms. In this definition, a low-complexity string has a higher probability of being generated by a randomly selected Turing machine, and therefore is less likely to have been produced by a random process (see development in Gauvrit et al., 2016). This has the double advantage of producing invariant estimates of complexity for a given observed sequence and that each experiment is treated as unique. In other words, each sequence is only compared with its own realization relative to the Turing machine algorithm. This method does not use thresholds to infer randomness (Zenil, 2015). Instead, it estimates the algorithmic complexity (AC) and an indicator of the computing time required to compress the sequence structure, called the logical depth (LD; Zenil et al., 2018). A longer LD means a non-trivial structure has been found in the sequence.

To apply this method, carapace vibration sequences produced by individuals during the same agonistic encounter were transformed to time-ordered, discrete binary series. Carapace vibrations were assigned to 1, if produced by the dominant individual, or 0 if produced by the submissive individual (e.g. 100000001010010); the rhythm of the carapace vibrations (i.e. the time between vibrations) and their duration were not represented. This also means we considered that two individuals produced carapace vibrations sequentially (i.e. as 'call-and-response') and not simultaneously. Because of the short length of our strings (from 14 to 98 characters), we used the block decomposition method made available through an online tool to access the necessary range of Turing machine states (Soler-Toscano et al., 2014; Zenil et al., 2018; <http://complexitycalculator.com/index.html>, v3.0). The most conservative settings were used: the largest available maximum block size (12), with no overlap and a two-character alphabet. As the AC and the LD both depend on string length, we report normalized values (as bits per character and steps per character, respectively). Hence, a standardized AC value of 1 or higher would be considered as not differentiable from random. Using a two-character alphabet, when the standardized LD is about 2 or higher, then the process that generated the sequences cannot be distinguished from a random one (Zenil et al., 2018).

Ethical note

Experiments with *H. gammarus* are not subject to restriction for animal scientific research according to the French legislation and the European Community Council Directive of September 2010 (2010/63/UE). We nonetheless followed the ARRIVE guidelines (Kilkenny et al., 2010) for all the experiments. The animals' health state was

checked daily by the authors and the aquariology team of Océanopolis. During experiments, we planned to stop the agonistic encounters between two lobsters before any injury occurred to the animals; this never happened and no lobsters were injured or died during the study. At the end of the experiments, all animals were released back into the area from where they were collected.

RESULTS

Description of the movements performed by male *H. gammarus* during agonistic encounters

When isolated on either side of the divided tank, lobsters wandered freely around the space and did not show any particular movements related to the other individual. When the divider was lifted, the individuals quickly engaged physically in an agonistic encounter (e.g. Fig. 2). Initially, they made a short (<1 min) series of threat displays, typically consisting of: antenna pointing or antenna whipping, claw open, meral spread and high on legs movements. Next, they advanced rapidly with different types of physical claw contact to drive away their opponent. This stage was mainly dominated by claw pushing movements. In 6 of the 12 agonistic encounters (4 with $\Delta CL > 5$ mm, 2 with $\Delta CL < 5$ mm), the outcome was decided at this stage. In the six other trials, the lobster pairs increased the intensity of the fight by using a variety of claw movements to attack their opponents. These movements, such as claw boxing, claw ripping or claw snapping, were very short in duration and occurred in association with aggressive upward-directed tail flipping. Generally, after these actions, one individual withdrew and assumed the submissive role for the remaining time (Fig. 2).

After this first encounter, which determined the hierarchical status between the two lobsters, each dominant and submissive individual displayed typical groups of movements (Fig. 2, Table 2). Dominant individuals continued to perform physical displays (i.e. meral spread, high on legs and claw open), and often approached the submissive individuals (i.e. walking toward) to re-engage in physical contact (mainly antenna whipping and claw pushing). In contrast, submissive individuals always responded by escaping through physically demanding movements such as walking backward and tail flipping (Fig. 2, Table 2). In particular, submissive individuals used a characteristic submissive posture with the claws extended in front of the animal for much of the period following the first encounter. Finally, when individuals were not making claw contact, the dominant animals were moving actively around the tank such as walking or sand removing, while in contrast, the submissive ones were relatively immobile (i.e. resting) near the tank walls with their claws extended (mean: 44.9% of time; Fig. 2, Table 2).

Buzzing sounds and carapace vibrations produced during agonistic encounters

During the agonistic encounters, we did not record any particular sounds other than the buzzing sounds with the hydrophones. We identified a total of 65 buzzing sounds from 9 of the 24 lobsters tested. In marked contrast, the accelerometer data showed that 23 out of the 24 lobsters tested vibrated their carapace during the agonistic encounters. The only lobster that did not vibrate its carapace was a dominant individual. From these 23 lobsters, a total of 422 carapace vibrations were recorded, meaning that only 15% of the associated buzzing sounds were recorded by the two hydrophones in the tank. Fig. 3 shows an example where two lobsters produced three carapace vibrations during a short period (6 s), and the associated buzzing sounds were only recorded by the closest hydrophone (<20 cm from the animals). However, in most other cases when

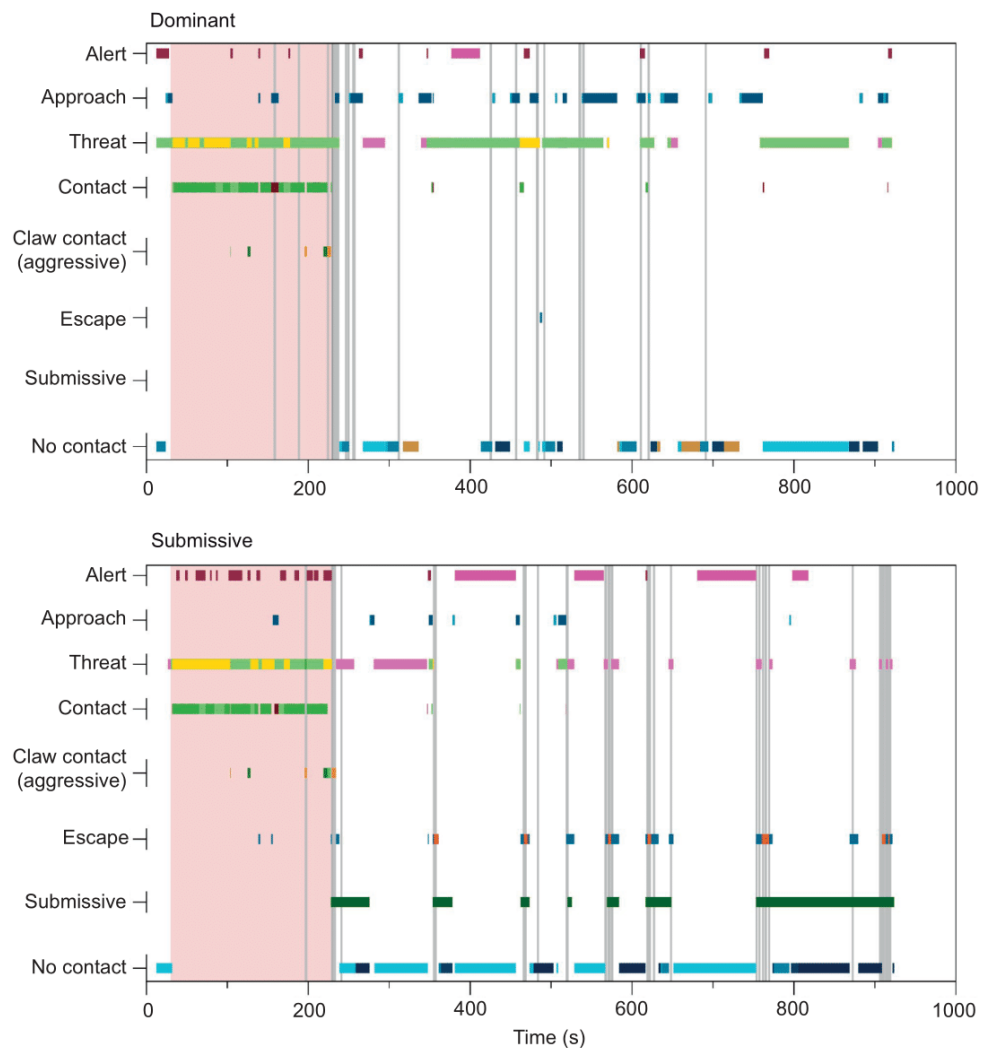


Fig. 2. Example chronology of movements (horizontal colour bars) performed by two lobsters during an agonistic encounter. Top, the dominant individual; bottom, the submissive individual (difference in carapace length, $\Delta\text{CL} < 5$ mm). The different bar colours refer to different movements performed by lobsters that are shown in Table 1 and Table 2. All movements were regrouped using the behavioural terms obtained in Table 2. The first encounter is shaded red. A total of 98 carapace vibrations (vertical grey bars) were produced by both lobsters during this experiment (dominant: 35; submissive: 63), whereas only 7 associated buzzing sounds (7.1%) were recorded by the two hydrophones. Note that most carapace vibrations were produced just after the first encounter and during episodes of approach/escape between dominant and submissive individuals.

lobsters vibrated their carapaces, the associated buzzing sounds were not recorded by the two hydrophones at the same time.

We therefore used the number of carapace vibrations as a proxy for the number of buzzing sounds produced by lobsters. No carapace vibrations were detected when lobsters were first separated from each other by the divider. Even though some first encounters were long (up to 3.38 min) with highly aggressive movements between lobsters (e.g. claw ripping), very few carapace vibrations (4.7%) were produced at this time (Fig. 2). In contrast, carapace vibrations were mostly (95.3%) produced after the first encounter (i.e. after hierarchical status was determined), during the stage of repeated approaches by the dominant individuals making threat displays towards the submissive individuals (Fig. 2).

Fig. 4 describes the distribution of all carapace vibrations detected by the accelerometers according to dominant or submissive outcomes. Overall, dominant individuals emitted about half as many carapace

vibrations as submissive ones (141 and 281, respectively). Carapace vibrations had durations that varied from about 50 ms to nearly 600 ms, and their peak frequencies varied between 100 and 200 Hz. No carapace vibrations were recorded that began at exactly the same time. These data were also plotted as time series for all 12 encounters (Fig. 5). There are few clear patterns in the series. The total number of carapace vibrations in an experiment between individuals of nearly the same CL (Fig. 5, left) tended to be higher than that in experiments where the ΔCL was > 5 mm (Fig. 5, right). Submissive individuals, which were also the only individuals to assume the 'extended claw' pose (Table 2), produced carapace vibrations in all encounters and mostly, but not always, while in this pose (Fig. 5). For most agonistic encounters, submissive individuals produced more carapace vibrations than did dominant ones; but in three experiments (Fig. 5C,H,L), the opposite pattern was obtained and the dominant animal vibrated more frequently. In one experiment, the dominant individual was silent (Fig. 5E).

Table 2. Overview of the movement assignments to behaviours and the time budgets for the 12 agonistic encounters

Behaviour	Movement annotated	Dominant (% time)	Submissive (% time)
Alert	Antenna sensing	4.2±6.2	17.3±14.2
	Antennae up	10.0±7.4	5.8±9.4
Approach	Turning toward	8.9±3.4	2.3±1
	Walking toward	25.7±7.3	3.8±2.5
Threat	Antennae backward	9.6±9.7	2.0±3.7
	Antenna pointing	20.2±8	21.9±16.9
	Claw forward	3.5±3.2	1.7±3.5
	Claws lunging	2.3±2	1.5±2.2
	Claw open	31.6±21.2	6.9±6.6
	High on legs	34.9±13.5	7.1±7.7
	Meral spread	35.3±11.3	2.8±9
Physical contact	Antenna touching	0.8±1.1	5.7±7.8
	Antenna whipping	11.7±7.8	2.1±3.2
	Claw pushing	7.8±6.5	3.9±5
	Claw touching	2.6±2.2	0.7±1.2
Aggressive claw contact	Claw boxing	0.1±0.2	0.1±0.1
	Claw grasping	0.3±0.9	0.2±0.4
	Claw locking	0.1±0.2	0.1±0.2
	Claw ripping	0.1±0.1	0.03±0.06
	Claw scissoring	0.1±0.1	0.1±0.2
	Claw snapping	0.02±0.06	0.05±0.1
	Tail flipping	0.2±0.3	2.4±2
Escape	Walking backward	1.0±0.8	18.9±5.6
	Claws extended	0.0±0	44.9±15.7
Submissive No contact	Facing	16.0±11.8	6.1±10.6
	Resting	12.3±6.7	44.8±11.7
	Sand removing	5.2±7.2	0.0±0
	Turning away	4.1±1.9	3.8±1.8
	Walking away	5.8±3.8	9.7±5
	Walking parallel	7.5±7.5	2.8±3

Mean (±s.d.) percentage of time in each movement is shown for the dominant and submissive individuals. Total time was 15 min for each encounter. Bold highlights significantly different means between dominant and submissive animals (U -test, $N_1=N_2=12$, $P<0.05$).

As described above, the carapace vibration series were then expressed as binary, ordered sequences and analysed for their AC and LD. The string standardized values of the AC and LD are given in Fig. 5, in bits per character and steps per character, respectively. The values of both measures ($1<AC<3$ and $2<LD<4$) indicate that the carapace vibration sequences were probably the product of a random process, and by themselves cannot be assimilated to call-and-response type signalling.

DISCUSSION

This study is the first report of male *H. gammarus* producing buzzing sounds during agonistic encounters. These sounds were produced by both dominant and submissive individuals during the experiments and were mainly emitted after the end of the first encounter (when claw contact stopped) up until the experiment ended.

Our agonistic encounters resembled descriptions of agonistic encounters published in earlier studies of male *H. americanus* (Scrivener, 1971; Atema and Voigt, 1995; Huber and Kravitz, 1995) and male *H. gammarus* (Skog et al., 2009). The initial stage consisted of a threat display between individuals that then quickly engaged in physical claw contacts, which could increase in aggressiveness (e.g. claw boxing) until the withdrawal of one individual (Fig. 2). This losing individual then exhibited submissive behaviours highlighted by a claws extended pose and was less active, while the winner remained active and continued to make approaches and threat displays. At the same time, both individuals produced buzzing sounds.

However, during these experiments, very few buzzing sounds were recorded by the two hydrophones even if they were placed close to the lobsters (<75 cm away). This is consistent with remarks made in previous studies on *H. americanus* (Scrivener, 1971; Atema and Cobb, 1980; Atema and Voigt, 1995; Ward et al., 2011). For example, Atema and Cobb (1980) stated that ‘the biological significance of such vibrations is unknown; during high intensity fights in aquaria, these sounds were rarely recorded’. Ward et al. (2011) showed, with accelerometry and sound recordings (as in this study), that the presence of another lobster in a tank significantly increased the number of buzzing sounds produced, but that these events were also rare (mean of 3 sounds per lobster in a 30 min experimental period). Nonetheless, these authors did not perform experiments concerned with agonistic behaviours between male individuals, and in addition, the accelerometers used in Ward et al. (2011) required that the lobsters were immobilized.

In the present study, we used small accelerometers which could be attached directly on the carapace where sound production occurs (Henninger and Watson, 2005). This unobtrusive sensor permitted the lobsters to exhibit their full range of agonistic movements. In contrast to the earlier studies, we found that the number of carapace vibrations recorded with the accelerometers was very high during agonistic encounters. Indeed, we recorded a total of 422 carapace vibrations produced by 23 out of the 24 lobsters tested, with some individuals producing up to 70 carapace vibrations per experimental period (15 min total). In contrast, only 15% of these carapace vibrations were picked up by the two hydrophones (e.g. Fig. 3). This difference in detection between hydrophones and accelerometers is explained by the high attenuation of low-frequency sounds in tanks. Although low frequencies are less attenuated than high frequencies in open water, the situation is reversed in tanks when the wavelength of the sound is larger than the tank size (e.g. a 100 Hz sound has a ~15 m wavelength). This phenomenon is well known in the acoustic community (Gray et al., 2016; Rogers et al., 2016), but sometimes misunderstood in the bioacoustic community. Nonetheless, it was recently highlighted through numerical simulations and empirical measurements. Indeed, Duncan et al. (2016) illustrated that the attenuation in a tank at 100 Hz is 10 dB higher than in open water (note that the exact number depends on the specific tank size and the source/receiver configuration). Moreover, Jézéquel et al. (2019) performed an empirical illustration of this phenomenon by comparing spiny lobster sounds in a tank and *in situ*. Because the high attenuation of low frequencies has been ignored in previous bioacoustic tank studies that relied on hydrophones alone, we believe that the role and importance of buzzing sounds for lobsters during agonistic encounters have been underestimated.

The detection or determination of communication amongst individual animals is a fundamental challenge in behavioural ecology (Hebets and Anderson, 2018). Communication is defined most simply as a transfer of information from one or more individuals that is observed to change the behaviour of one or more receiving individuals. Information can be transmitted and perceived in many different ways (e.g. chemically, visually, acoustically) depending on the sensory capabilities of the organisms involved. Several studies have already shown that male *H. americanus* use chemical signals as a means of communication to both recognize individuals and maintain dominance (Atema and Engstrom, 1971; Karavanich and Atema, 1991; Breithaupt and Atema, 2000). These same mechanisms are also known for male *H. gammarus* (Skog et al., 2009). Studies have demonstrated that the volume of urine

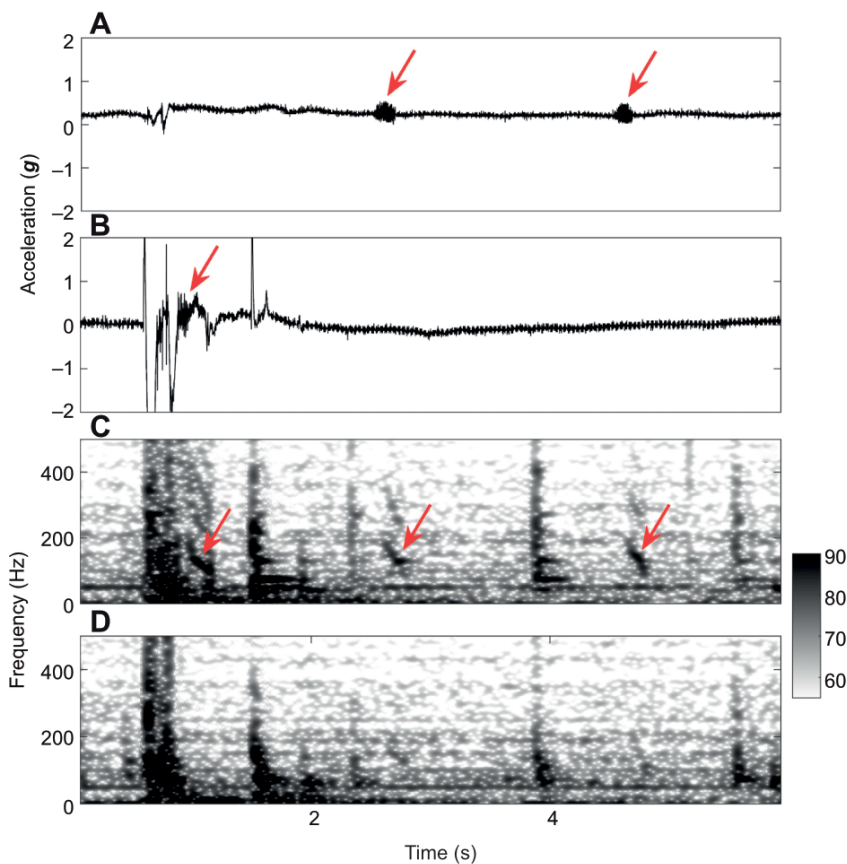


Fig. 3. Synchronized data of two accelerometers (top) and the two hydrophones (bottom) during an agonistic encounter. The red arrows highlight the carapace vibrations and the associated buzzing sounds. The dominant lobster (CL=13 cm) used claw contact and produced two carapace vibrations (A) toward the submissive lobster (CL=11.3 cm), which escaped by tail flipping (large negative peaks) and produced one carapace vibration (B). The three associated buzzing sounds were recorded by the hydrophone located at <20 cm from the animals (C) while the other more-distant hydrophone (D) did not record them. The vertical colour bar scale of the spectrograms is in dB re. $1 \mu\text{Pa}^2 \text{Hz}^{-1}$.

released is closely linked with aggressive behaviours (Breithaupt et al., 1999) and, after the first encounter, only dominant individuals continue to release urine to maintain their dominance (Breithaupt and Atema, 2000). There is also evidence that lobsters rely on visual signals to recognize each other (Gherardi et al., 2010; Bruce et al., 2018). All these means of communication emphasize the importance of individual-level recognition of submissive and dominant individuals. For example, this would be an advantage for avoiding additional aggressive claw contact incidents that could lead to injuries and even loss of an appendage (Breithaupt and Atema, 2000).

Dominance among male lobsters also relies on their relative size differences (Scrivener, 1971). In our study, 6 out of the 12 agonistic encounters were performed with closely size-matched pairs ($\Delta\text{CL} < 5 \text{ mm}$). As the encounters studied here represent examples of possible outcomes of new arrival dominance contests and not repeat encounters, the conditions should be suitable for a more important role of other signals conditioning the outcome, particularly for the encounters with closely size-matched pairs. In accordance with this, there were some intriguing patterns in the production of carapace vibrations. Indeed, we observed that

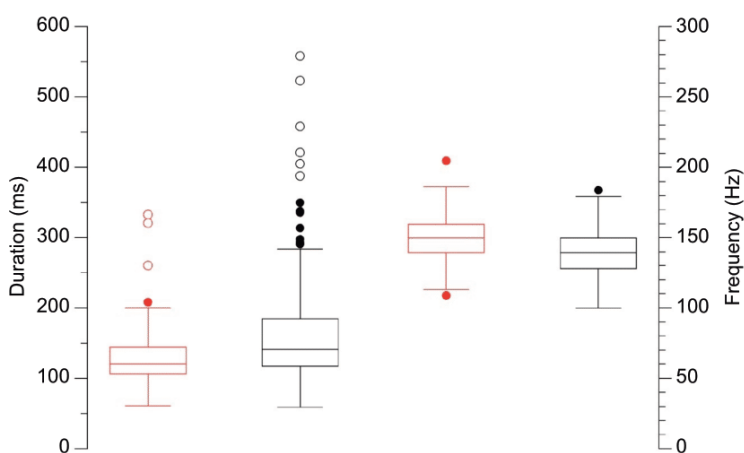


Fig. 4. Box plots of carapace vibration features produced by dominant (red) and submissive (black) animals during agonistic encounters. Median values for each group are indicated by the horizontal lines. Left: duration; right: frequency.

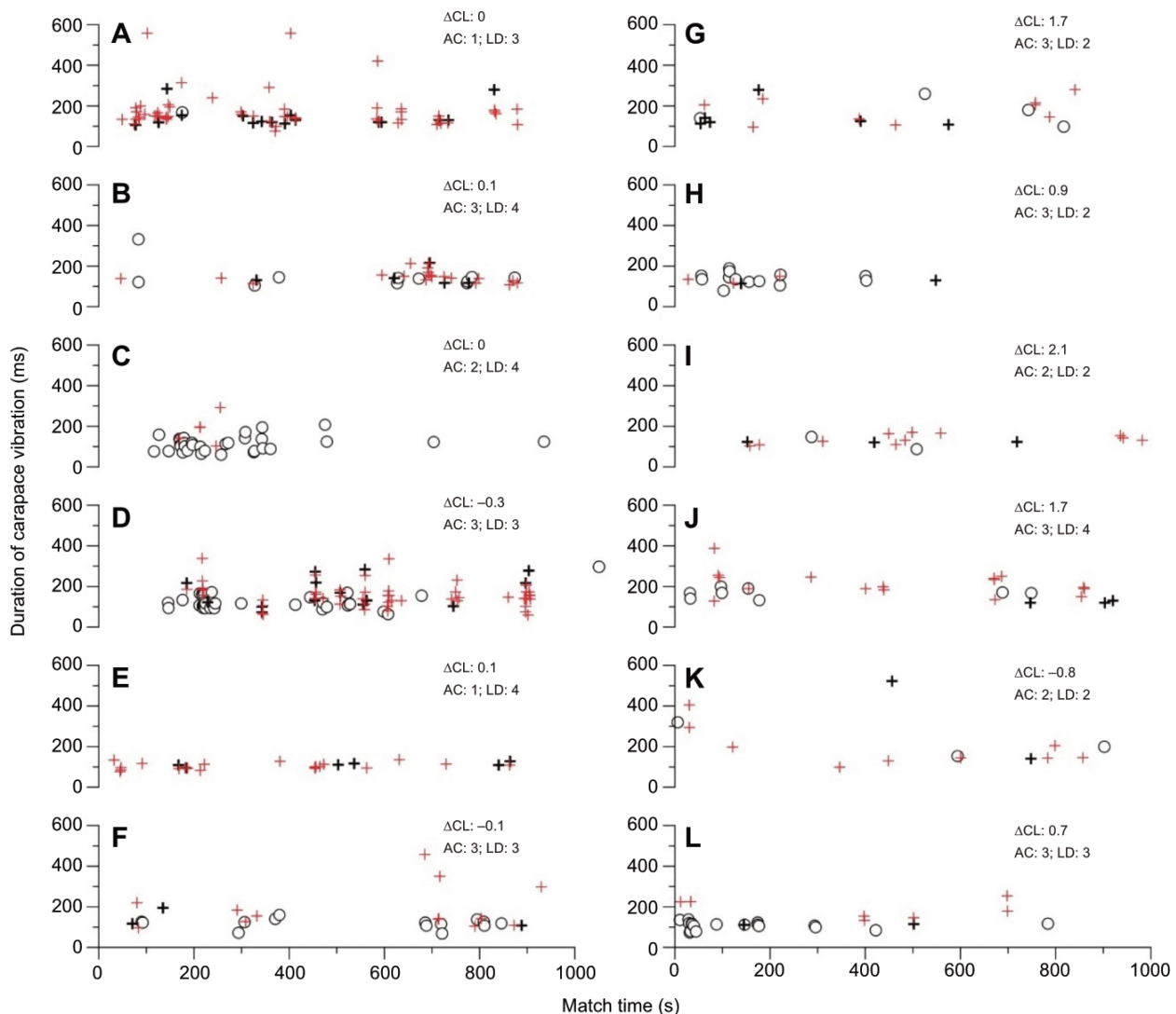


Fig. 5. Emitted carapace vibrations and their duration for all 12 agonistic encounters. Dominant animals (open circles) mostly vibrated less than submissive ones (crosses). Red crosses indicate when the submissive individuals vibrated while in extended claw pose. On each plot, the relative difference in CL (ΔCL) is indicated for each pair (estimated as the difference in length of the dominant and submissive individual); the left column of plots is for the $\Delta CL < 5$ mm size class and the right column is for the $\Delta CL > 5$ mm size class. The ratios of the algorithmic complexity (AC) and logical depth (LD) standardized by the string length are also given. In our analysis, all the values indicate that the pattern of vibrations emitted by the two individuals during the encounters cannot be differentiated from a random process.

submissive individuals always produced carapace vibrations, and these mostly occurred while in the claws extended pose, as well as having a broader range of duration and a higher number of carapace vibrations produced (Figs 4 and 5). In contrast, dominant individuals did not always produce a carapace vibration (e.g. Fig. 5E). We also noted that no carapace vibrations were produced by lobsters while isolated before agonistic encounters. However, examination of the carapace vibration sequences using the paradigm of AC (Gauvrit et al., 2016; Zenil et al., 2015preprint, 2018) indicated that these sequences cannot be differentiated from a random process. As stated earlier, this could be due to their non-detection by lobsters because of the high attenuation of these low-frequency sounds in the tanks (Gray et al., 2016; Rogers et al., 2016).

When looking at sequences of carapace vibrations between the two groups of encounters with different relative CLs, the more

closely size-matched pairs ($\Delta CL < 5$ mm) appeared to make a greater investment in countering the strategies of their opponents. Indeed, these encounters had more carapace vibrations, which implied more effort expended to counter the opponent's reactions (Fig. 5). These preliminary results are consistent with the hypothesis that carapace vibration sequences in pairs of nearly sized-match individuals contribute to the communication of dominance, but that when size differences are larger, other signals (i.e. visual) are sufficient to establish dominance (Scrivener, 1971; Atema and Voigt, 1995; Skog et al., 2009). Interestingly, such multimodal communication is well known in terrestrial arthropods (e.g. Elias et al., 2006). However, we caution that as stated above, the vibration sequences cannot be distinguished from a random process and that there is a potential bias due to sound attenuation in tanks, as well as a small number of observations.

Our results emphasize not only the numerous technical challenges in these experiments but also the absence of knowledge about how lobsters may perceive sounds. For instance, in our study, the lack of a call-and-response pattern with carapace vibrations between lobsters was surprising. Indeed, individuals only produced vibrations when in the presence of a potential opponent, strongly suggesting their emission is context dependent. If combinatoriality (that is, the property of constructing meaning from apparently meaningless elements) is present, then the acoustic production would be considered communication if it can be shown to provoke a predictable response (Engesser and Townsend, 2019). This highlights the need to better understand how animals perceive sounds to be able to design appropriate experiments.

Lobsters cannot directly detect pressure from buzzing sounds, but they may still be able to detect the corresponding particle motion (Breithaupt and Tautz, 1990; Breithaupt, 2001; Popper et al., 2001). Indeed, a large diversity of sensory receptors dedicated to this function is known in both *H. americanus* and *H. gammarus*, including statocysts and sensory hairs (Cohen, 1955; Laverack, 1962). By considering this, Breithaupt (2001) suggested that lobsters may only be able to detect these sounds in the near-field, i.e. at distances less than a few tens of centimetres from the source. This hypothesis is consistent with the close-range communication well described in terrestrial arthropods (Raboin and Elias, 2019). Here, we did not measure or model the acoustic particle motion field in the behavioural area as this was out of the scope of the study. As a result, if the lobsters were unable to detect the buzzing sounds using particle motion, we do not know whether this is due to the specificities of tank acoustics and/or because of biological reasons. Validating (or rejecting) this hypothesis would require further work, including model and/or measurement of near-field particle motion of lobster buzzing sounds (active and reactive intensity; e.g. Zeddies et al., 2012; Jones et al., 2019), and a better understanding of the lobster sound perception system (Breithaupt, 2001).

While some studies have confirmed experimentally the role of sound production in marine crustaceans to deter predators (Bouwma and Herrnkind, 2009; Ward et al., 2011), few studies have demonstrated these sounds are used for intraspecific communication. Interestingly, stomatopods produce low-frequency sounds termed ‘rumbles’ that are similar to the lobster buzzing sounds (Patek and Caldwell, 2006; Jézéquel et al., 2018). Mantis shrimps are territorial species living in burrows, like lobsters, and their sounds might help to send signals of their presence to conspecifics to maintain territory (Staaterman et al., 2011). Spiny lobsters have also been shown to emit antennal rasps during agonistic encounters in tanks (Mulligan and Fischer, 1977), suggesting that these sounds may be used as a threat display. Snapping shrimps may also use their powerful ‘snaps’ to deter other conspecifics from their territory (Schmitz and Herberholz, 1998). During agonistic encounters, male hermit crabs produce rapping sounds by rubbing their claws against their carapace, which may be a signal of stamina (Briffa et al., 2003). In marked contrast to other marine crustacean species where behavioural responses to sounds are not yet clear, semi-terrestrial crabs (Ocypodidae) have been shown not only to produce sounds (e.g. Taylor et al., 2019) but also to respond to these sounds during intraspecific interactions (Crane, 1966; Horch and Salmon, 1969; Horch, 1975).

In our earlier study (Jézéquel et al., 2018), we found that *H. gammarus* produced buzzing sounds when stressed by handling. In the present study, agonistic encounters led to stressful events for both dominant and submissive individuals that resulted in the production of buzzing sounds. Thus, these sounds may be used in a similar context to the spiny lobster antennal

rasps and the mantis shrimp rumbles to repel other organisms, whether conspecifics or heterospecifics (Mulligan and Fischer, 1977; Bouwma and Herrnkind, 2009; Staaterman et al., 2011). Taken together, these preliminary results suggest that male *H. gammarus* could use buzzing sounds, in addition to visual and chemical signals (Skog et al., 2009), as a means of intraspecific communication during agonistic encounters. However, we emphasize that our study should be repeated and include additional tests to evaluate whether these buzzing sounds really constitute communication. Other experiments should test behavioural reactions to emitted sounds as well as build an audiogram for the species associated with the quantification of particle motion (Goodall et al., 1990; Popper and Hawkins, 2018). As shown in this study, because small tanks highly attenuate buzzing sounds, these experiments should be done under controlled conditions or directly in the field (Gray et al., 2016; Rogers et al., 2016). This would also be expected to change the behavioural observations. Indeed, it is not yet known at what frequency and intensity lobsters fight for dominance under *in situ* conditions where escape is possible (Karnofsky et al., 1989). Finally, additional studies should address the acoustic behaviour of female lobsters during agonistic encounters, as they have also been shown to be aggressive towards conspecifics (Skog, 2009).

Conclusion

In this study, we have highlighted for the first time that male *H. gammarus* produce buzzing sounds during agonistic encounters. Notably, we showed that they only emitted sounds when placed in contact with each other, and that most of these sounds were produced after the first encounter (i.e. hierarchical status had been determined). However, we did not find clear evidence that these sounds could be used for communication between individuals. This may be due to the high attenuation of the buzzing sounds in tanks, which could prevent their perception by receivers. Other studies have suggested that these buzzing sounds could be a means of intraspecific communication in lobsters (Breithaupt and Tautz, 1990; Breithaupt, 2001). Further studies are now needed to validate this new hypothesis.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: Y.J., J.C.-G., L.C., J.B.; Methodology: Y.J., J.C.-G., L.C., J.B.; Software: J.B.; Validation: J.C.-G., J.B.; Formal analysis: Y.J.; Data curation: Y.J., J.C.-G., J.B.; Writing - original draft: Y.J., J.C.-G., J.B.; Writing - review & editing: Y.J., J.C.-G., L.C., J.B.; Visualization: Y.J., J.C.-G.; Supervision: J.C.-G., L.C., J.B.; Project administration: L.C.; Funding acquisition: L.C.

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Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.211276.supplemental>

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Revisiting the bioacoustics of European spiny lobsters *Palinurus elephas*: comparison of antennal rasps in tanks and *in situ*

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ABSTRACT: Spiny lobsters (Palinuridae) are capable of emitting sounds called antennal rasps. In the bioacoustics literature, such broadband sounds have mostly been characterized from tank recordings where reverberation and resonant frequencies might strongly distort their features. Hence, in this study, we compared antennal rasps produced by European spiny lobsters *Palinurus elephas* in both tank and *in situ* conditions. We found significant differences in all sound features (temporal, intensity and spectral features) between tank and *in situ* recordings, confirming that antennal rasps — and broadband sounds generally — cannot be accurately characterized in tanks if sound reverberation is ignored. In recordings of antennal rasps made *in situ*, we show that the main acoustic power is located in the low frequency band (below 1 kHz), which was missed by all earlier studies done in tanks where such low frequencies cannot be properly measured. The hearing capacities of crustaceans suggest roles for intra-specific communication of these sounds, and their high levels indicate they could be heard above noise. Indeed, we outline that antennal rasps are among the loudest sounds known in the marine animal kingdom, with peak-to-peak sound pressure levels (calculated at 20 cm from the source) above 175.7 dB re 1 μPa^2 , and peak-to-peak source levels (estimated at 1 m from the source) ranging from 154.2 to 160.6 dB re 1 μPa^2 . These acoustic properties imply they could be detected *in situ* during passive acoustic monitoring. This study also highlights the importance of using appropriate measurement methods when characterizing sounds produced by marine invertebrates.

KEY WORDS: Passive acoustics · Spiny lobsters · Palinuridae · *Palinurus elephas* · Antennal rasp · Tank reverberation · Resonant frequencies

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1. INTRODUCTION

Ecologists are looking for new environmental descriptors to monitor marine ecosystems. Among the different tools available to survey coastal ecosystems, passive acoustics seems promising, mostly because it is non-invasive and non-destructive (Rountree et al. 2006). Recent studies have highlighted the potential of passive acoustics to monitor tropical marine eco-

systems by focusing on producers of specific sounds, such as snapping shrimps and fish (Lammers et al. 2008, Kaplan et al. 2015, Deichmann et al. 2018). In contrast, temperate marine ecosystems have received much less attention, and there is a growing interest to identify species producing sounds that can be detected and isolated among ecosystem soundscapes. In addition to accurately characterizing sounds emitted by these species, we also need to be able to asso-

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ciate them with particular behaviors to understand their ecological meanings (Briffa et al. 2003, Ladich & Myrberg 2006). These steps are crucial before envisaging the use of biological sounds as potential marine ecosystem indicators. Crustaceans should be considered as having high potential for bioacoustics studies because they emit a large diversity of sounds (Staaterman 2016), particularly in temperate coastal waters (Coquereau et al. 2016).

Among marine crustaceans, tropical spiny lobsters have received attention for many decades in marine bioacoustics because they produce specific sounds (Moulton 1957, Meyer-Rochow & Penrose 1976, Patek et al. 2009). The mechanism of sound production is external, is located at the base of each second antenna and consists of 2 parts: the soft, ridged plectrum that rubs posteriorly over the anterior part of the hard file-like surface covered with microscopic shingles (Meyer-Rochow & Penrose 1974, Patek 2001, Patek & Baio 2007). This stick-and-slip movement generates a series of broadband pulses during each slip. The associated sound produced during a full movement is composed of many short pulses (the pulse train), and each pulse train is called an 'antennal rasp' (Moulton 1957, Hazlett & Winn 1962b, Patek et al. 2009). The ecological meaning of antennal rasps has mostly been attributed to an anti-predator context, such as helping clawless spiny lobsters to deter or escape from predators (Bouwma & Herxkind 2009, Staaterman et al. 2010). However, far less is known about the bioacoustics of spiny lobsters living in temperate coastal waters (Buscaino et al. 2011a,b).

The European spiny lobster *Palinurus elephas* (Fabricius, 1787) inhabits European coastal waters from the Northeast Atlantic to the Mediterranean Sea (Ceccaldi & Latrouite 2000) and occurs from the shoreline down to about 200 m water depth (Hunter 1999). It is a large, mobile crustacean with an activity pattern characterized by diurnal sheltering and nocturnal foraging (Giacalone et al. 2015). Its life cycle is quite different from other benthic crustaceans, because it has an unusually long pelagic larval stage (between 6 and 12 mo) followed by benthic juvenile and adult stages, with growth happening through successive molts (Hunter 1999). Because of its high commercial value, *P. elephas* has been historically overfished in many European waters (Goñi & Latrouite 2005), which brought this species to its current status of 'Vulnerable' on the IUCN Red List (Goñi 2014). A striking example of population collapse is found in Brittany, France, where landings dropped from 1000 t in the 1950s to <20 t by the

2000s (Goñi & Latrouite 2005). In this context, it is critical to find new study tools, such as passive acoustics, to monitor this species in these areas.

Antennal rasps produced by spiny lobsters, including *P. elephas*, have mostly been investigated and characterized in tank experiments (e.g. Hazlett & Winn 1962a, Mulligan & Fischer 1977, Buscaino et al. 2011a; see Table S1 in the Supplement at www.int-res.com/articles/suppl/m615p143_supp.pdf). These studies in tanks are necessary before performing *in situ* recordings, because they permit isolating the sounds produced by the studied species, without ambiguity from other sources of sounds present in the oceans. Tank studies are also useful to make visual observations that are essential to associate sounds with behaviors. However, sound characterization in tanks presents many challenges, particularly for broadband sounds that are commonly produced by crustaceans. In a previous article, Jézéquel et al. (2018) illustrated that reverberation in small, constrained volumes like the tanks often used in marine bioacoustics studies, extends the sound duration. In addition, the resonant frequencies, which are specific to each tank's dimensions (Akamatsu et al. 2002), distort the spectral shape of broadband sounds so that their intensity and spectral features cannot be characterized properly (Jézéquel et al. 2018). Moreover, low-frequency sounds (i.e. with frequency below the first resonant frequency of the tank used) are highly attenuated and cannot be measured properly (Rogers et al. 2016). Thus, broadband sounds, such as antennal rasps produced by spiny lobsters, should not be characterized in tanks without a full understanding of the sound reverberation in the tanks. Ideally, tank recordings should be complemented by *in situ* recordings, which mitigate the reverberation issue, while introducing other experimental difficulties (e.g. visibility, ambient noise). Accurately characterizing a sound is important to be able to detect it among the myriad other biological sounds present in marine environments (e.g. Tricas & Boyle 2014, Putland et al. 2017), and is also critical for inferring its potential ecological role.

Here we characterized for the first time the antennal rasps emitted by the European spiny lobster *P. elephas* under *in situ* conditions. We first compared different features of these sounds calculated from both an experimental tank and *in situ*, and then compared them with the existing literature. We assessed the potential ecological roles of these sounds based on our results and determined the potential of such sounds for *in situ* passive acoustic monitoring.

2. MATERIALS AND METHODS

2.1. Ethical statement

Experiments with European spiny lobsters are not subject to restriction for animal scientific research according to the French legislation and the European Community Council Directive of September 2010 (2010/63/UE). However, we followed the ARRIVE guidelines (Kilkenny et al. 2010) to ensure that all experiments were performed under good conditions. Animals were handled with care during the study, and their health status was checked daily by the authors and the aquariology staff of the public aquarium Océanopolis in Brest, France, where the laboratory experimental work was done. No specimens were harmed during this study and there was no mortality. At the end of the study, all specimens were released back into the environment where they were collected.

2.2. Antennal rasp recordings and video

Sounds were recorded using 1 pre-amplified hydrophone (HTI-96-MIN, High Tech), with a sensitivity of -163.7 dB re $1 \mu\text{Pa}^{-1}$ and a flat response from 2 Hz to 50 kHz. This hydrophone was connected to a Wildlife Acoustics Song Meter (Model SM2) recorder with a gain of 0 dB, and was powered by cell batteries. Recordings were made with a sampling frequency of 48 kHz at a 16-bit resolution. The HTI-96-MIN / Wildlife SM2 recording device had the capacity to record sounds with sound pressure levels in peak-to-peak up to 185 dB re $1 \mu\text{Pa}^2$. This is crucial to characterize powerful pulses without clipping the recorded sound (i.e. sound saturation).

Video recordings were made during laboratory and *in situ* experiments using 2 GoPro® HERO3 cameras at a recording rate of 29.97 frames s^{-1} . The videos permitted confirmation of antennal rasp production by the spiny lobsters tested.

2.3. Laboratory experiment

2.3.1. Animal collection, characteristics and care

The laboratory work was carried out at the Océanopolis public aquarium in Brest. For these experiments, a total of 13 *Palinurus elephas* juveniles (7 males and 6 females) were used. Specimens had a carapace length (CL), as measured from the anterior

tip of the rostrum to the medial point of the posterior carapace margin, between 4.2 and 7 cm. They were collected carefully by hand while scuba diving in the Bay of Brest, at depths of between 15 and 25 m during 2 diving sessions on 18 and 19 July 2018. Only inter-molt individuals with full sets of intact appendages were selected for this study. After capture, they were immediately transferred randomly to 4 glass-sided rectangular holding tanks ($0.6 \times 0.5 \times 0.35$ m, length \times width \times effective height; seawater volume = 0.105 m^3), with 2 to 4 individuals per holding tank. Each individual was described (size, sex) and given an identification number.

Holding tanks were continuously supplied with sand-filtered, UV-sterilized seawater pumped from the Bay of Brest. Temperature, salinity and animal conditions were controlled twice a day. During the holding period, temperature varied between 17.08 and 18.15°C, and salinity between 34.84 and 35.05. All animals were fed with fresh pieces of fish (mackerel) and cephalopods (squid) ad libitum. They were kept under a 12:12 h light:dark photoperiod; daylight conditions (from 08:00 to 20:00 h) were simulated using fluorescent light tubes above the holding tanks. Several sections of rigid PVC pipe were provided as shelters in each tank. The bottom of each holding tank was supplied with a layer of sand 5 cm deep to provide a suitable foothold surface for the animals. Animals were acclimatized at least 1 mo in these holding conditions before they were used in experiments.

2.3.2. Experimental set-up and conditions of antennal rasp recordings

For the laboratory recordings, we used a plastic-sided rectangular tank ($1.13 \times 0.73 \times 0.5$ m; 0.4 m^3) which was placed in a quiet room. During the experiments, it was continuously supplied with the same seawater as the holding tanks, and the bottom was also covered with clean sand. One video camera (same model GoPro® as mentioned above) was placed on the bottom of the tank along the center of the short wall, and a second one was placed 50 cm above the center of the tank, looking down (i.e. plan view). During recordings, the tank was lit with 2 LED strips placed 50 cm above the water surface.

Before antennal rasp recordings, the flow of seawater in the room was cut off. The ambient noise of the experimental tank was recorded for at least 10 min without the animals to check for any additional electrical or other transient sounds. Spectrum analysis of

the ambient noise in the experimental tank showed the absence of any peak frequencies compared to those that were present during sound recordings made with animals. At low frequencies (≤ 50 Hz), peaks present corresponded to electrical noise from the recorder. These peaks were excluded from the analyses of biological sounds.

Next, we carefully transferred 1 spiny lobster from its holding tank to the experimental tank and waited for the individual to stop exploring and assume a resting position (about 5 min). The animal was then gently picked up and held at distances of between 20 and 30 cm from the hydrophone and about 20 cm off the substrate to elicit antennal rasps, as is commonly described in the bioacoustics literature on spiny lobsters (Moulton 1957, Meyer-Rochow & Penrose 1976, Patek et al. 2009). Antennal rasp recordings lasted about 30 to 60 s (individuals stopped emitting antennal rasps after this period), and all individuals were tested once during the study. After recordings were complete, each individual was returned to its holding tank, and sound files were archived for analysis.

2.4. *In situ* recordings

2.4.1. Site description

For comparison, we also recorded antennal rasps from spiny lobsters *in situ*, in the Bay of Perros Guirec, Brittany, France, where small groups of juveniles have been observed by recreational divers. Three different spots were selected in the bay, within 3 km of each other, where small groups of individuals were present. Juveniles were observed to be in typical habitats (similar to habitats described in the Mediterranean Sea; Díaz et al. 2001) of vertical, rocky outcrops (approximately 5 to 10 m high), with less than 12 m seawater depth at low tide. They were covered with colonies of jewel anemones (genus *Corynactis*) and colonial gorgonians (*Eunicella verrucosa*) just below the kelp belt (*Laminaria digitata*). These outcrops have many crevices inhabited by juvenile spiny lobsters. Because these areas are often subject to strong water currents (>2 knots), we performed antennal rasp recordings 30 min before and after low tide, when tidal currents were lowest.

2.4.2. *In situ* recordings of antennal rasps

Antennal rasp recordings were made on 29 September, and again on 21 October 2018 at the sites

described above. We deployed the recording device about 2 m from the rock face and at 2 m above the substrate, in front of the crevices where spiny lobsters were visible. First, the ambient noise at each site was recorded for 10 min. Just before sounds were recorded, 2 experienced divers free-dived to explore the vertical rocky outcrops and note the different locations where spiny lobsters were found. This permitted us to observe and determine individually the different spiny lobsters that could be tested, to avoid testing the same lobster twice. After this, we first filmed and then carefully collected an individual spiny lobster and brought it quickly to within 20 to 50 cm of the hydrophone, and 10 m below the seawater surface. During the manipulation, the spiny lobster was held so that it faced the hydrophone, and pointed away from the rocky face. The individual was then gently released back into its crevice. All individuals that were successfully caught were tested once, and each antennal rasp recording session lasted around 30 to 60 s. Carapace length and sex for each tested individual were both noted. Because the antennal rasp recordings were performed while free-diving, background noise from the diving activity was minimal in the vicinity of the recording device.

2.5. Sound analysis

2.5.1. Sound features of antennal rasps

Synchronized recordings of sounds (in .wav format) and videos were analyzed to confirm antennal rasp production by the spiny lobsters tested. Based on this annotation, each antennal rasp was extracted manually using the Audacity software® (version 2.1.1; Audacity Team 2015). Antennal rasps were defined as pulse trains composed of several pulses separated by less than 20 ms from each other. Hence any isolated pulses (mostly present in the *in situ* recordings) were not analyzed here. All sequences were then processed using custom MATLAB scripts (version 9.1; The MathWorks). Antennal rasps from tank recordings were analyzed between 60 Hz and 24 kHz to exclude electrical noise; frequencies below 60 Hz were ignored but not filtered. On the other hand, antennal rasps from *in situ* recordings were analyzed between 0 Hz and 24 kHz. No infra-sounds related to electronic self-noise from recording devices used were present during *in situ* recordings.

Antennal rasps from tank and *in situ* recordings were characterized by examining 3 different types of sound features: temporal, intensity and spectral fea-

tures. The different sound features are presented as mean \pm SD.

For tank and *in situ* recordings, we calculated 3 different temporal features: total duration (in ms), number of pulses per antennal rasp and pulse rate (in Hz). Total duration was calculated as the duration between the first 'rise' of the first and the last pulses of each antennal rasp (Jézéquel et al. 2018). This allowed us to avoid some of the effects of reverberation that extended each single pulse duration in the experimental tank. The pulse rate was defined as the number of pulses per train divided by the total duration. When pulses from an antennal rasp could not be clearly isolated from each other (i.e. because the reverberation of one pulse overlapped with the next one, or because 2 antennal rasps occurred simultaneously), we did not calculate its temporal features.

We also calculated 2 different intensity features based on sound pressure level (SPL, in dB re $1 \mu\text{Pa}^2$). As antennal rasps are pulse trains characterized by short and transient sounds, we chose to calculate the SPL in peak-to-peak (SPL_{pp}) which is the most representative intensity feature for these types of sounds (Erbe 2010). We also calculated the SPL as a root mean square (SPL_{rms}) that can be applied to pulse trains if they are homogeneous in time and long enough, so that they look like continuous signals at the scale of the analysis window.

In addition, for antennal rasps recorded *in situ*, source levels were estimated as peak-to-peak (SL_{pp}) at 1 m from the spiny lobsters, based on SPL_{pp} and a model of transmission loss (TL). As the hydrophone was located near the sound source (i.e. <1 m from the spiny lobsters), we assumed the antennal rasps propagate uniformly in all directions, permitting the use of a simple spherical spreading loss model to account for TL (Erbe 2010). Thus, SL_{pp} was estimated as follows: $\text{SL}_{\text{pp}} = \text{SPL}_{\text{pp}} + \text{TL}$, with $\text{TL} = 20\log_{10}(r)$ where r indicates the distance (in meters) between the spiny lobster and the hydrophone. Because it was difficult to maintain spiny lobsters underwater at a constant distance from the hydrophone during antennal rasp recordings while free-diving, we estimated the r from the videos as a minimum and maximum distance between the individual tested and the hydrophone. We estimated these distances between the spiny lobster held by the first diver and the hydrophone through the video recordings done by the second diver, and by knowing the dimensions of the recording device. The second diver filmed perpendicular to the direction of the spiny lobster held near the hydrophone, so that the Pythagorean theorem could be applied to calculate these distances. We then obtained 2 different peak-

to-peak source levels: SL_{pp} min and SL_{pp} max. Note that in this study, the *in situ* SL_{pp} estimates ignored any near-field effect. Because the use of more complex models of TL should be applied for antennal rasps recorded in the experimental tank, and because those models require a very accurate estimation of the source-receiver distance (Rogers et al. 2016), we did not estimate their SL_{pp} .

We also calculated 3 different spectral features: The first and second peak frequencies (Fp_1 and Fp_2 , respectively; in kHz) represent the 2 frequencies where the power spectral density (PSD, in dB re $1 \mu\text{Pa}^2 \text{Hz}^{-1}$) was maximal. These frequencies for antennal rasps recorded in the experimental rectangular plastic tank were compared with the minimum resonant frequency of the tank. This minimum resonant frequency (in kHz) was calculated using the theoretical equation from Akamatsu et al. (2002) for a rectangular glass-sided tank with mode (1, 1, 1). Finally, the frequency bandwidth (B , in kHz) was estimated as the spread (i.e. standard deviation) of the PSD around Fp_1 .

2.5.2. Ambient noise characterization

Recordings of ambient noise (10 min each) from the 5 *in situ* spots were first visualized to ensure the absence of antennal rasps. Next, they were both cut into 10 sequences of 1 min each, and we randomly selected 3 of 10 sequences from each recording per site. Ambient noise is characterized as a continuous sound (Erbe 2010); we thus calculated the SPL_{rms} of each selected, 1 min long sequence. This provided a mean value of the SPL_{rms} for the ambient noise at each spot. We also generated the sets of theoretical Wenz curves (Wenz 1962) for boat traffic noise (ranging from index 1 to 7) and wind noise (ranging from 0 to 30 knots) using custom MATLAB scripts. They were used as a comparison for the PSDs of the ambient noise calculated at each spot.

2.6. Statistical analysis

We first tested whether differences in means between the carapace length of individuals tested between the 2 groups (tank and *in situ*) were significant. As these data were normally distributed in both groups (Shapiro-Wilk test, $p < 0.05$), Student's *t*-test was applied ($\alpha = 0.05$).

All sound features described above (except for the SL_{pp} that was only calculated for antennal rasps re-

corded *in situ*) were then tested to determine whether significant differences in means were evident between the 2 groups. We aimed to compare tank and *in situ* recordings of antennal rasps produced by *P. elephas* using the same handling protocol. Thus, no variability among antennal rasps produced by a single individual, nor between individuals could be considered from the data collected. Considering the small number of sound recordings, and assuming that calculated variables for each individual can be assimilated to a random distribution, the non-parametric Mann-Whitney *U*-test was used to determine if their probability distributions were equal ($\alpha = 0.001$). All statistical analyses were performed using R version 3.5.1 (R Core Team 2018).

3. RESULTS

3.1. Sound features of antennal rasps

In both tank and *in situ* recordings, all spiny lobsters tested produced audible antennal rasps during episodes of stress generated while handling. These were mostly accompanied by visible, vigorous contractions of their abdominal regions.

3.1.1. Tank recordings

A total of 387 antennal rasps from the 13 individuals tested (21–34 rasps ind.⁻¹) were recorded in the experimental tank. Waveforms consisted of pulse trains that were sometimes difficult to isolate because of sound reverberation (Fig. 1A). The effects of sound reverberation were also seen in spectrograms where all pulses (dark vertical lines) were followed by a ‘smear’ (dark horizontal lines; Fig. 1C). When taking this into account, temporal features could be calculated in 94% of the antennal rasp events. Their mean total duration was 120.5 ± 25.9 ms (range: 60–225 ms), with a mean of 15.0 ± 3.3 pulses per antennal rasp (range: 7–28) and a mean pulse rate of 127.9 ± 21.1 Hz (range: 78.4–226.7 Hz) (Table 1).

Intensity features were high, with a mean SPL_{pp} of 171.0 ± 3.1 dB re $1 \mu Pa^2$, including some antennal rasps recorded at 20 cm from the spiny lobsters of up to 175.7 dB re $1 \mu Pa^2$ (Table 1). The mean SPL_{rms} was 151.2 ± 4.2 dB re $1 \mu Pa^2$ (Table 1).

The minimum resonant frequency calculated for the experimental tank was 1.94 kHz, and strongly influenced the spectral shape of the antennal rasps. Indeed, 268 out of the 387 (69%) antennal rasps had

their first peak frequencies at 2 kHz, which was clearly seen in both the PSDs (Fig. 1B) and spectrograms (dark horizontal line at 2 kHz; Fig. 1C). Below 2 kHz, a relatively large acoustic power gap was found in all antennal rasps (Fig. 1B,C). In contrast, above 2 kHz, high acoustic power was present up to 23 kHz (Fig. 1B,C). These higher frequencies corresponded to other resonant frequencies associated with the experimental tank and caused a highly variable F_{p1} and F_{p2} , with means of 3.99 ± 3.68 kHz (range: 1.82–17.74 kHz) and 5.34 ± 4.27 kHz (1.82–17.83 kHz), respectively (Table 1). Because acoustic power was mainly focused around the minimum resonant frequency of the tank, the resulting bandwidth was limited, with a mean of 5.13 ± 2.51 kHz (0.42–11.70 kHz) (Table 1).

3.1.2. *In situ* recordings

During *in situ* recordings, 9 spiny lobsters (6 females, 3 males) were successfully caught and their antennal rasps recorded in the 3 different spots described above. Their mean CL was 5.1 ± 0.4 cm (SD) and did not differ significantly from the mean CL of spiny lobsters used for the laboratory experiments (*t*-test, $p = 0.32$). A total of 233 antennal rasps were recorded from all 9 spiny lobsters (range: 17–32 rasps ind.⁻¹).

Compared to antennal rasps recorded in the experimental tank, antennal rasps recorded *in situ* presented clear waveforms with pulses that could be easily isolated (Fig. 1D). This was also highlighted in the spectrograms, which did not have the smearing seen in the tank recordings (Fig. 1C,F). Thus, temporal features could be calculated for all 233 recorded antennal rasps emitted by the 9 individuals tested. Mean total duration was 147.0 ± 29.7 ms (53–266 ms), with a mean number of pulses per antennal rasp of 16.9 ± 4.7 (6–33) and a mean pulse rate of 115.9 ± 27.2 Hz (59.4–208.9 Hz; Table 1). Mean temporal features from antennal rasps recorded *in situ* were all significantly different from the antennal rasps recorded in the tank; the mean total duration and mean number of pulses per antennal rasp were higher (*U*-test, $p < 0.001$), whereas the mean pulse rate was lower (*U*-test, $p < 0.001$).

As seen in Table 1, even if some SPL_{pp} and SPL_{rms} values were also high (up to 175.7 and 146.4 dB re $1 \mu Pa^2$ at 20 cm from the spiny lobsters, respectively), their mean values were significantly lower than those measured in the experimental tank (*U*-test, $p < 0.001$). The mean values of SL_{pp} estimated at 1 m

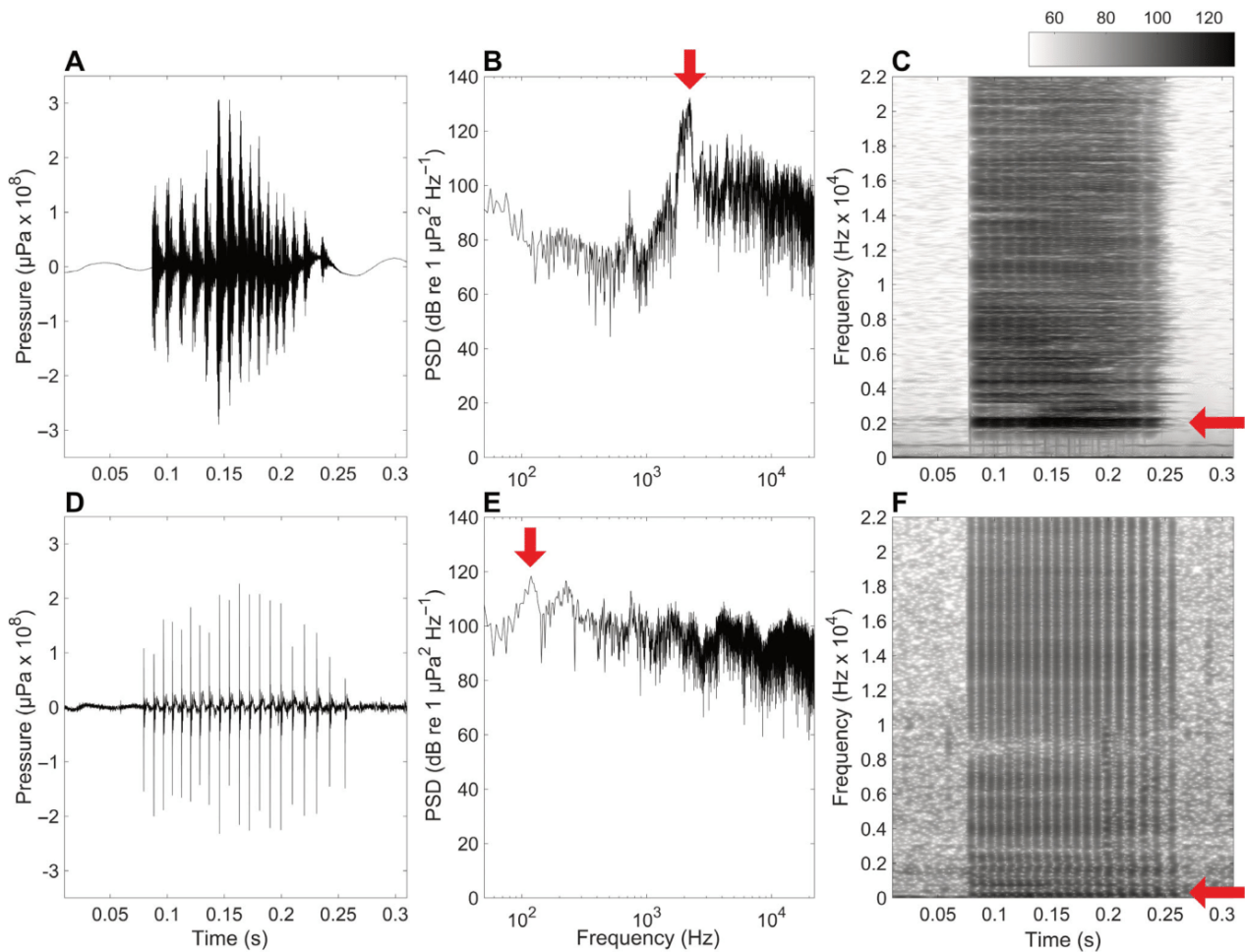


Fig. 1. Examples of typical antennal rasps produced by 2 *Palinurus elephas* juveniles and recorded at the same distance from the hydrophone (20 cm) in the experimental tank (top row) and *in situ* (bottom row). (A,D) Waveforms. (B,E) Acoustic spectra (Fast Fourier Transform length: 13640), with the x -axis in logarithmic scale. (C,F) Spectrograms (Fast Fourier Transform length: 2024; Hamming window: 1001 points; 99% overlap). Red arrows indicate the first peak frequency of each antennal rasp. Notice that the first peak frequency of the antennal rasp recorded in the tank (top row) corresponds to the minimum resonant frequency calculated for the experimental tank alone (2 kHz); there is also a large gap of acoustic power below 2 kHz. The color scale bar is in dB re $1 \mu\text{Pa}^2 \text{Hz}^{-1}$. PSD: power spectral density

from the spiny lobsters in the field ranged between 154.2 ± 4.5 ($\text{SL}_{\text{pp min}}$) and 160.6 ± 4.4 ($\text{SL}_{\text{pp max}}$) dB re $1 \mu\text{Pa}^2$ (Table 1).

As no effects of reverberation were detected for the antennal rasps recorded *in situ*, we could calculate their natural spectral features for the first time. Acoustic power peaks were found to be spread in the low frequency band (below 1 kHz; Fig. 1E,F). Indeed, mean values of Fp_1 and Fp_2 were respectively 0.77 ± 0.24 kHz (0.12–1.66 kHz) and 0.96 ± 0.40 kHz (0.22–1.62 kHz) (Table 1). These were both significantly lower than the tank recordings (U -test, $p < 0.001$). This is the contrary to what was seen in the tank recordings where acoustic power was only found

from 2 kHz (Fig. 1B,C). However, these low peak frequencies did not include all the acoustic power contained in antennal rasps, with some acoustic power also found in higher frequencies (up to 23 kHz). This gave a mean bandwidth of 16.99 ± 5.38 kHz (4.90–23.00 kHz; Table 1) that was significantly larger than that of the tank recordings (U -test, $p < 0.001$).

3.2. Comparison of ambient noise and antennal rasps recorded *in situ*

During *in situ* recordings, the sea state was between 1 (Calm) and 2 (Smooth) on the Douglas scale,

Table 1. Sound features (temporal, intensity and spectral features) calculated for the European spiny lobster antennal rasps recorded in the experimental tank and *in situ*. Results are presented as means \pm SD with ranges (minimum–maximum). A Mann-Whitney test was used to compare means of sound features between the tank and *in situ* recordings. **Bold** values highlight sound features that were significantly different (*U*-test, $p < 0.001$) between tank and *in situ* recordings. SPL_{pp} (SPL_{rms}): peak-to-peak (root mean square) sound pressure level, SL_{pp}: peak-to-peak source level, Fp₁ (Fp₂): first (second) peak frequency, *B*: frequency bandwidth, NA: not applicable

Sound features	Antennal rasps	
	Tank	<i>In situ</i>
Temporal		
Total duration (ms)	120.5 \pm 26.0 (60–225)	147.0 \pm 29.7 (53–266)
Number of pulses per antennal rasp	15.0 \pm 3.3 (7–28)	16.9 \pm 4.7 (6–33)
Pulse rate (Hz)	127.9 \pm 21.1 (78.4–226.7)	115.9 \pm 27.2 (59.4–208.9)
Intensity		
SPL _{pp} (dB re 1 μ Pa ²)	171.0 \pm 3.1 (160.4–175.7)	167.3 \pm 3.9 (156.0–175.7)
SPL _{rms} (dB re 1 μ Pa ²)	151.2 \pm 4.2 (139.7–159.6)	139.2 \pm 3.0 (132.0–146.4)
SL _{pp} min (dB re 1 μ Pa ²)	NA	154.2 \pm 4.5 (142.0–165.3)
SL _{pp} max (dB re 1 μ Pa ²)	NA	160.6 \pm 4.4 (150.0–169.7)
Spectral		
Fp ₁ (kHz)	3.99 \pm 3.68 (1.82–17.74)	0.77 \pm 0.24 (0.12–1.66)
Fp ₂ (kHz)	5.34 \pm 4.27 (1.82–17.83)	0.96 \pm 0.40 (0.22–1.62)
<i>B</i> (kHz)	5.13 \pm 2.51 (0.42–11.70)	16.99 \pm 5.38 (4.90–23.00)

corresponding to wave heights of between 0 and 0.5 m. Wind state ranged between 0 (Calm) and 4 (Light breeze) on the Beaufort scale, corresponding to speeds between 1 and 18 knots. Seawater temperature was $16.5 \pm 0.2^\circ\text{C}$ and salinity was 35.4 ± 0.1 .

Two different groups of ambient noise were distinguished among the 5 different spots where sound recordings were made. Two spots were directly subject to more energetic sea conditions (high wind speeds and waves), resulting in mean SPL_{rms} values of 121.0 ± 0.5 and 124.2 ± 1.0 dB re 1 μ Pa². In contrast, the 3 other locations were calmer, which was reflected in a lower mean SPL_{rms} of 109.5 ± 1.6 dB re 1 μ Pa². During ambient noise recordings, no antennal rasps were detected.

The PSD of the loudest ambient noise had its main acoustic power peaks at more than 100 dB re 1 μ Pa² Hz⁻¹ below 25 Hz that was attributed to the friction of the hydrophone with water due to the waves (Fig. 2). The ambient noise levels at all 5 locations were within Wenz curves of between 25 Hz and 2 kHz. Above 2 kHz, acoustic power peaks were always found around 3 and 4 kHz that were almost 20 dB re 1 μ Pa² Hz⁻¹ above Wenz curves (Fig. 2). This contribution was associated with isolated broadband pulses from unknown sources (no snapping shrimps were observed at the spots during the recordings). Despite these observations, PSDs of all antennal rasps recorded at the 5 spots were always above the PSDs of the ambient noise recorded at the same time and over the entire frequency range (except at frequencies below 25 Hz). For example, the PSD of a typical antennal rasp at its first peak frequency (0.125 kHz) was 30 dB re 1 μ Pa² Hz⁻¹ above the PSD of the loudest ambient noise, and almost 50 dB re 1 μ Pa² Hz⁻¹ above the quietest (Fig. 2). This pattern was also seen in the higher frequencies up to 23 kHz (Fig. 2).

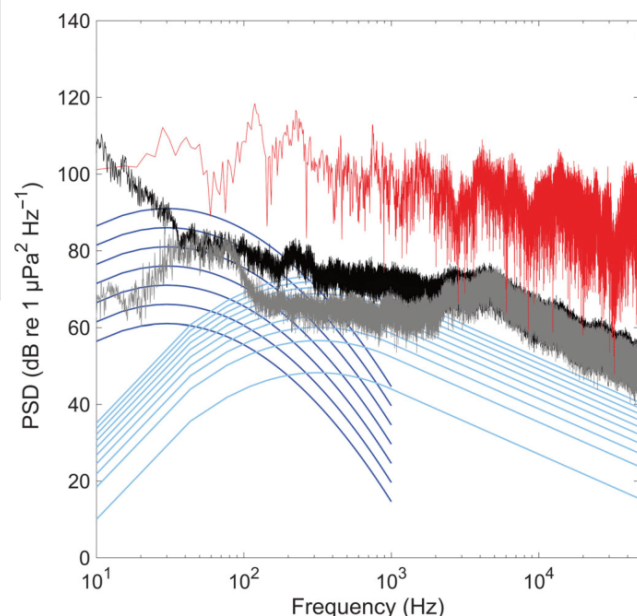


Fig. 2. Acoustic spectrum of a *Palinurus elephas* antennal rasp recorded *in situ* (red), with the loudest ambient noise (black) and the quietest ambient noise (grey) recorded from the 5 different spots. The x-axis is in logarithmic scale. Thin lines indicate Wenz's traffic noise curves ranging from index 1 to 7 (dark blue) and Wenz's wind noise curves ranging from 0 to 30 knots (light blue). PSD: power spectral density

4. DISCUSSION

In this study of *Palinurus elephas* antennal rasps, we emphasize 2 important results. First, all sound features calculated between antennal rasps recorded in an experimental tank and *in situ* were significantly different. Secondly, this is the first time that *P. elephas* antennal rasps have been characterized *in situ*. We have shown that they are very loud, with SPL_{pp} values (calculated at 20 cm from the source) being higher than 175.7 dB re 1 μPa^2 and SL_{pp} values (estimated at 1 m from the source) ranging from 154.2 to 160.6 dB re 1 μPa^2 , and with important acoustic power spread in low frequencies (below 1 kHz). These new results suggest possible ecological roles for these sounds and a strong potential for them to be detected *in situ*.

4.1. Temporal features of the antennal rasps

Sound reverberation in the experimental tank did affect the waveform of antennal rasps by extending the duration of single pulses (Fig. 1A,C). This phenomenon was absent in antennal rasps recorded *in situ* (Fig. 1D,F). Our results for temporal features are consistent with antennal rasps previously reported for *P. elephas* in the bioacoustics literature. Our mean total durations of 120.5 and 153.1 ms (tank and *in situ* recordings, respectively) were within an order of magnitude of other studies (Patek & Oakley 2003: 101 ms; Buscaino et al. 2011a: 90 ms; de Vincenzi et al. 2015: 70 ms), and are similar to antennal rasp total durations previously published for tropical spiny lobsters (Mulligan & Fischer 1977: *Panulirus argus*, 154 ms; Patek & Oakley 2003: *Panulirus japonicus*, 155.1 ms). In our study, the mean numbers of pulses per antennal rasp recorded in the experimental tank and *in situ* are in the same range as reported for studies done on *P. elephas* (Patek & Oakley 2003: 13.5; Buscaino et al. 2011a: 9.5; de Vincenzi et al. 2015: 8) and other tropical spiny lobsters (Patek & Oakley 2003: *Linuparus trigonus*, 17.9; Kikuchi et al. 2015: *Panulirus japonicus*, 11). Finally, our mean pulse rates of antennal rasps recorded in the experimental tank and *in situ* are close to other studies on *P. elephas* (Patek & Oakley 2003: 138.5 Hz; Buscaino et al. 2011a: 118.2 Hz; de Vincenzi et al. 2015: 134 Hz), and other species of tropical spiny lobsters (Patek & Oakley 2003: *L. trigonus*, 118.5 Hz; *Justitia japonica*, 131.4 Hz; *Panulirus homarus*, 120.6 Hz).

Even though we recorded and compared 2 groups of juveniles with similar sizes by using the same pro-

cedure, we found significant differences in all temporal features between tank and *in situ* recordings. This was surprising, as the temporal features we calculated are not impacted by tank reverberation. This variability could be attributed to differences between individuals and also between antennal rasps produced by the same individual or even the behavioral context (handling); however, these were not investigated for this explanatory study. For example, long-legged spiny lobsters *Panulirus longipes* show fatigue after producing antennal rasps for more than 30 s (Meyer-Rochow & Penrose 1976). Fatigue or exhaustion would be expected to lower the rates of antennal rasps and change their temporal features (e.g. pulse rate reduced; Meyer-Rochow & Penrose 1976).

Several authors working on tropical spiny lobsters have examined the scaling of antennal rasp temporal features with body size and even the mechanism of sound production. For example, Patek & Oakley (2003) found positive correlations between pulse rate and the number of pulses with file length, while antennal rasp total durations were negatively correlated with file length for *Panulirus argus*. Meyer-Rochow & Penrose (1976) and Patek et al. (2009) found a positive correlation between body size and antennal rasp total durations, while there was a negative correlation between size and pulse rate for *Panulirus longipes* and *Panulirus interruptus*, respectively. In this study, we used only similar-sized individuals. Performing additional sound recordings using a wider size range could permit verification of antennal rasp temporal features and the importance of inter-individual variability.

4.2. Intensity of antennal rasps

This is the first time that such high SPL values for antennal rasps have been reported in the bioacoustics literature on spiny lobsters. We calculated antennal rasp SPL_{pp} values at more than 175.7 dB re 1 μPa^2 in the tank and *in situ* at 20 cm from the specimens tested. In addition, because we recorded antennal rasps *in situ*, we could estimate for the first time their SL_{pp}. We found the SL_{pp} estimated at 1 m from the spiny lobsters ranged between 154.2 and 160.6 dB re 1 μPa^2 . However, 2 different intensity features (SPL_{pp} and SPL_{rms}) were significantly lower in the *in situ* recordings compared to tank recordings. For example, the mean SPL_{rms} of antennal rasps *in situ* was 10 dB re 1 μPa^2 lower than in the tank recordings. This difference in SPL_{rms} is explained by

the 'smear of noise' accompanying each pulse in all antennal rasps recorded in the tank that increased sound intensity (see Fig. 1A,C), and this phenomenon was absent in antennal rasps recorded *in situ* (see Fig. 1D,F). In addition, due to the low-frequency sampling of our recording device (48 kHz), these intensity features might be underestimated if there is acoustic power spread at higher frequencies.

Several articles have reported SPLs in decibels for spiny lobsters (Latha et al. 2005, Patek et al. 2009, Buscaino et al. 2011a, de Vincenzi et al. 2015); the numerical values in the literature range from 50 to 150. Unfortunately, these results are not comparable because they were obtained with different signal-processing methods, and these earlier studies do not provide enough information to be able to re-estimate the values. For example, calculating an SPL on a frequency representation produces different numerical results depending on the method applied (e.g. power spectrum or PSD) and on the calculation parameters (e.g. sampling frequency, Fast Fourier Transform length). It should also be noted that antennal rasps are short, transient sounds. Fourier-based methods are poorly adapted for such signals, for the reasons previously discussed. As stated in ANSI (2005) and demonstrated by Erbe (2010), it is preferable to use time-domain metrics such as the SPL_{pp} and SPL_{rms} calculated in our study. Thus, here we chose to compare our results with other studies that also reported intensity features in SPL and SL from sounds emitted by other marine species.

Few marine animals have been reported to emit such loud sounds. Some marine mammals, such as dolphin and whale species, have been shown to produce whistles and calls with SL_{pp} at 1 m that exceed 169 and 189 dB re $1 \mu Pa^2$, respectively (Janik 2000, Sirovic et al. 2007). Some fish have also been shown to emit very loud sounds, including large goliath groupers *Epinephelus itajara* with a maximum SPL_{rms} of 144 dB re $1 \mu Pa^2$ (distances from the fish not estimated; Mann et al. 2009), or black drums *Pogonias cromis* with highest SPL_{rms} calculated at 0.95 m exceeding 166 dB re $1 \mu Pa^2$ (Locascio & Mann 2011). In crustaceans, only snapping shrimps *Alpheus heterochaelis* have been shown to produce louder impulsive sounds (the 'snaps') compared to the antennal rasps of *P. elephas*. Snaps have been calculated to have SL_{pp} estimated at 1 m ranging from 145 to up to 215 dB re $1 \mu Pa^2$ (Cato & Bell 1992, Au & Banks 1998, Schmitz 2002). In the present study, we only recorded antennal rasps in juveniles (CL between 4 and 7 cm); larger adults (CL > 15 cm) may emit even louder antennal rasps if the earlier results of Meyer Rochow

& Penrose (1976) for the evolution of antennal rasp features with body size in *Panulirus longipes* can be generalized.

4.3. Spectral features

In our study, spectral features of antennal rasps recorded in the experimental tank were strongly affected by the tank resonant frequencies. Indeed, acoustic power was only present from 2 kHz (the minimum resonant frequency of our tank) to 23 kHz. This distribution of acoustic power toward high frequencies in our recordings is generally consistent with the bioacoustics literature on spiny lobsters. Buscaino et al. (2011a) and de Vincenzi et al. (2015) calculated both very high and variable first peak frequencies of 19.52 ± 6.70 and 22.93 ± 8.20 kHz, respectively, for *P. elephas* antennal rasps recorded in tanks. Other studies (also done in tanks) for species of tropical spiny lobsters also found high peak frequencies in antennal rasps (e.g. Hazlett & Winn 1962a, Mulligan & Fischer 1977, Patek et al. 2009; see Table S1). For example, antennal rasps produced by *Panulirus argus* in a tank of similar size to the tank used in the present study showed their first peak frequencies between 2 and 5.5 kHz (Mulligan & Fischer (1977), which should have been described as being associated with the tank resonant frequencies (Akamatsu et al. 2002, Jézéquel et al. 2018). Meyer-Rochow & Penrose (1976) were the first authors to mention the technical challenges of characterizing antennal rasps in tanks because of sound reverberation and resonant frequencies. They reported that antennal rasps were distorted by these phenomena, hence they did not calculate spectral features of the sounds (peak frequencies and bandwidth). These authors concluded that antennal rasps should contain acoustic power spread equally over their bandwidth (i.e. a white spectrum; Meyer-Rochow & Penrose 1976). In contrast, below 2 kHz, we found a large acoustic power gap that was present in all antennal rasps recorded in the experimental tank (Fig. 1B,C). This gap below the minimum resonant frequency of our experimental tank is explained by the high attenuation of low frequencies in such tanks because of their longer wavelength compared to the dimensions of the tank used (Rogers et al. 2016).

Not surprisingly, spectral features of antennal rasps recorded *in situ* showed significantly different patterns compared to antennal rasps recorded in the experimental tank. Indeed, the important acoustic power was present in the low frequency band (below

1 kHz; Table 1). These results are representative of the true antennal rasp sound, as our *in situ* measurements were not contaminated by tank reverberation. Thus, our results do not confirm the hypothesis of a white spectrum postulated by Meyer-Rochow & Penrose (1976). However, our results are consistent with the later work of Patek et al. (2009) using *Panulirus interruptus*, where these authors reported the F_{p1} in antennal rasps recorded *in situ* as 0.63 ± 0.37 kHz. In addition, Moulton (1957) described antennal rasps from *Panulirus argus* with peak frequencies of 0.80 kHz in a large naturalized aquarium. However, Kikuchi et al. (2015) recorded antennal rasps *in situ* from *Panulirus japonicus*, with mean values of peak frequencies of 10.00 ± 4.50 kHz. While we used the same protocol as in the existing bioacoustics literature in spiny lobsters to have comparable results, we did not take into account the potential effects of animal handling in the spectral features of antennal rasps. Further studies will be required to characterize antennal rasps without handling animals *in situ*. An interesting perspective is the use of fake predators, as described by Staaterman et al. (2010).

In addition, the bandwidths of our antennal rasps recorded *in situ* were significantly broader compared to antennal rasps recorded in the tank (Table 1). This is due to the minimum resonant frequency of the tank that concentrated the acoustic power, leading to distinct peaks in the PSDs (Fig. 1B). In contrast, spectra of antennal rasps recorded *in situ* had acoustic power spread much wider around peak frequencies (Fig. 1E). However, the bandwidth values reported here were limited by our system capacity. Because of the sampling frequency (48 kHz), we could not record frequencies higher than 24 kHz. Indeed, the estimated bandwidth (up to 23 kHz) actually covered the whole bandwidth of the recording device, and thus we concluded that the source bandwidth might be greater than 23 kHz. As a confirmation, Buscaino et al. (2011a) found acoustic power up to 100 kHz in antennal rasps produced by *P. elephas* in tanks.

4.4. Towards a new biological hypothesis concerning the potential ecological roles of antennal rasps in *P. elephas*

Accurately characterizing a sound produced by a particular species is not only crucial to be able to detect it *in situ* among other biological sounds, but also for inferring biological hypotheses about their potential ecological roles. In the next sections, we discuss the implications of our results.

4.4.1. Inter-specific communication

Most studies in the bioacoustics literature have proposed an anti-predator role for the antennal rasps (Moulton 1957, Meyer-Rochow & Penrose 1976, Staaterman et al. 2010). In this study, we elicited the production of antennal rasps in *P. elephas* by gently handling individuals, which is intended to imitate capture by a natural predator. In contrast with the clawed lobster *Homarus gammarus*, the clawless *P. elephas* relies on its spiny carapace and rigid antennas to defend itself against predators (Barshaw et al. 2003). Thus, being able to emit loud sounds could help individuals to deter potential predators. In contrast with previous studies (Buscaino et al. 2011a, de Vincenzi et al. 2015), we have demonstrated that most of the acoustic power contained in antennal rasps was spread into the low frequency band below 1 kHz. Interestingly, the sound sensitivity of some of their natural predators, namely cephalopods (including octopus), appears to be limited to the same band of low frequencies (Williamson 1988, Packard et al. 1990, Mooney et al. 2010). For example, a controlled experiment with *Panulirus argus* showed that stridulating individuals escaped more frequently from attacking octopuses than surgically muted individuals (Bouwma & Herrnkind 2009). In addition, Buscaino et al. (2011a) found that *P. elephas* produced more antennal rasps when exposed to an octopus than in the trials without an octopus in a tank. As the common octopus *Octopus vulgaris* is a well-known predator of the European spiny lobster (Barshaw et al. 2003) and is typically found in Brittany coastal waters in the same areas inhabited by *P. elephas* juveniles, these antennal rasps may indeed serve as a deterrent.

Other taxa could also be affected by this sound. Several fish species have been described as predators of tropical spiny lobsters, including triggerfish of the genus *Balistes* (Briones-Fourzán et al. 2006, Lavalli & Herrnkind 2009). The bioacoustics literature has shown that most fish hear best below 1 kHz (Ladich & Popper 2004). Thus, even though the triggerfish audiogram has not been described yet, it is likely that it could also hear in this low frequency band. Bouwma (2006) suggested that antennal rasps produced by *Panulirus argus* against triggerfish is aposematic, advertising the lobster's spiny defenses to predators. Interestingly, the tropical gray triggerfish *B. capricus* is now a common species in Brittany coastal waters (Quéro et al. 2008) and would encounter *P. elephas*.

Finally, and by examining the spectral features of antennal rasps recorded *in situ*, the large bandwidth

(mean of 16.99 kHz) can provide a clear evolutionary advantage in clawless spiny lobsters. It seems possible that those types of broadband sounds could have been selected because they permit their emitters to target a maximum of different potential receivers (i.e. potential predators). Even molted spiny lobsters with a soft carapace can still produce antennal rasps, an observation which has also been reported for the buzzing sounds produced by the European lobster *H. gammarus* (Jézéquel et al. 2018). Thus, even when their protective carapace is weakened, the antennal rasps could still function as a deterrent (Patek 2001).

4.4.2. Intra-specific communication

Few studies have hypothesized a potential intra-specific communication of antennal rasps in spiny lobsters. This could be explained by our observation that most bioacoustics studies have characterized antennal rasps in tanks and reported that acoustic power is only present in high frequencies (above 10 kHz; e.g. Buscaino et al. 2011a), which could not be detected by crustaceans (Goodall et al. 1990, Popper et al. 2001, Lovell et al. 2005). Our findings now allow us to suggest how these sounds could be used as a means of intra-specific communication.

Crustaceans lack gas-filled organs, like swim bladders, required for pressure detection, but may be still capable of detecting low-frequency acoustic stimuli arising from the second component of acoustic energy, i.e. particle motion (Breithaupt & Tautz 1990, Popper et al. 2001, Popper & Hawkins 2018). For example, the Norway lobster *Nephrops norvegicus* responds *in situ* to particle motion over a frequency range of 20–200 Hz (Goodall et al. 1990). In addition, Lovell et al. (2005) found that common prawns *Palaemon serratus* in tanks are sensitive to particle motion generated by low-frequency sounds ranging from 100 Hz to 3 kHz. Diverse sensory receptors, adapted to detect particle motion from low-frequency sounds, have been described in crustaceans, including statocysts and sensory hairs (Popper et al. 2001, Radford et al. 2016). In this study, we did not measure the particle motion arising from antennal rasps *in situ*. However, as the pressure component associated with antennal rasps is very high, it is likely that the associated particle motion would also be elevated (Nedelec et al. 2016). Additional studies are now required to measure and understand the potential directionality of particle motion/sound generated by these antennal rasps *in situ* (Popper & Hawkins 2018). We also need to quantify the detection bandwidth and thresholds

(i.e. audiogram) in *P. elephas* through other means, such as behavioral studies (Goodall et al. 1990).

Spiny lobsters, and particularly juveniles such as those used in this study, are gregarious and generally live in high densities in shelters (Atema & Cobb 1980). Several studies have found that they use chemical and visual signals to maintain a social status inside a group of conspecifics through the use of aggressive agonistic encounters (Zimmer-Faust & Spanier 1987, Ratchford & Eggleston 1998, Shabani et al. 2009). Our results imply that antennal rasps may also be involved. Interestingly, Berrill (1976) showed that post-plerulus larvae of *Panulirus longipes* use agonistic encounters when competing for limited access to food and shelter, and that they use antennal rasps during these stressful events as a threat display.

4.5. Antennal rasp detection in underwater soundscapes

In contradiction with the existing bioacoustics literature, our study has highlighted the high acoustic potential of antennal rasps produced by spiny lobsters *in situ*. Our results clearly suggest that antennal rasps produced by *P. elephas* could be detected *in situ* above the ambient noise. Surprisingly, earlier studies carried out with tropical spiny lobsters *Panulirus interruptus*, using the same protocol of sound recordings as in this study, stated that antennal rasps recorded *in situ* are as loud as the ambient noise, so that they could not be detected (Patek et al. 2009, Staaterman et al. 2010). As discussed in Section 4.2, because the use of metrics to characterize sounds is not normalized (and sometimes missed), it is not possible to do a direct comparison of our results with those of Patek et al. (2009). Nonetheless, our ambient noise measurements (PSD, in dB re 1 $\mu\text{Pa}^2 \text{Hz}^{-1}$) are fully consistent with other measurements performed in the same area (Mathias et al. 2016, Kinda et al. 2017).

Conversely, our hypothesis of antennal rasp detection *in situ* is consistent with the work of Kikuchi et al. (2015) who recorded *in situ* antennal rasps from *Panulirus japonicus*. These authors found a positive correlation between the frequency of detected antennal rasps and the number of spiny lobsters caught in nets, and also showed an increase in detected antennal rasps during night time. The European spiny lobster *P. elephas*, similar to *Panulirus japonicus*, has an activity pattern characterized by diurnal sheltering and nocturnal foraging (Giacalone et al. 2015). During nocturnal movements, *P. elephas* individuals could encounter other conspecifics or predators, leading to

the production of antennal rasps that would be detectable with hydrophones. However, further studies are needed to validate this potential method of detection and the distance of sound propagation at potential monitoring locations with high lobster densities.

5. CONCLUSIONS

We have revisited the bioacoustics of *Palinurus elephas* and more generally that of spiny lobsters as a group. First, we have confirmed that broadband sounds, such as the antennal rasps, cannot be accurately characterized in tanks (except for the temporal features used in this study) because of sound reverberation and tank resonant frequencies. Indeed, the tank properties distort shapes of broadband sounds. In contrast, antennal rasps from spiny lobsters can be characterized accurately when recorded directly in their natural environment (*in situ*). We have provided 3 different types of sound features: temporal, intensity and spectral features. Secondly, we have shown for the first time that antennal rasps are among the loudest sounds produced among marine animals. We have also highlighted that their acoustic power is present in a low frequency (below 1 kHz); such low-frequency content was missed in previous studies that were conducted in tanks. Using these new results, we could suggest a new biological hypothesis concerning their ecological roles, in particular for intra-specific communication, that has been overlooked in the bioacoustics literature. Finally, we clearly demonstrated that these sounds have suitable properties for *in situ* passive acoustic monitoring. This could contribute to additional *in situ* behavioral studies to better understand *P. elephas* movement patterns. This could also be developed as a tool for the management of *P. elephas* fisheries, especially to permit detection of areas with juveniles in order to protect them from destructive fishing practices (Goñi & Latrouite 2005).

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Spiny lobster sounds can be detectable over kilometres underwater

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The detection ranges of broadband sounds produced by marine invertebrates are not known. To address this deficiency, a linear array of hydrophones was built in a shallow water area to experimentally investigate the propagation features of the sounds from various sizes of European spiny lobsters (*Palinurus elephas*), recorded between 0.5 and 100 m from the animals. The peak-to-peak source levels (SL, measured at one meter from the animals) varied significantly with body size, the largest spiny lobsters producing SL up to 167 dB re 1 μ Pa². The sound propagation and its attenuation with the distance were quantified using the array. This permitted estimation of the detection ranges of spiny lobster sounds. Under the high ambient noise conditions recorded in this study, the sounds propagated between 5 and 410 m for the smallest and largest spiny lobsters, respectively. Considering lower ambient noise levels and different realistic propagation conditions, spiny lobster sounds can be detectable up to several kilometres away from the animals, with sounds from the largest individuals propagating over 3 km. Our results demonstrate that sounds produced by *P. elephas* can be utilized in passive acoustic programs to monitor and survey this vulnerable species at kilometre scale in coastal waters.

Passive acoustic monitoring (PAM) of marine species has recently gained attention by biologists and is now used worldwide. This is due to the increased knowledge of animal sound repertoires, and the behavioral contexts in which they are produced^{1–3}. In addition, the density of seawater enables sounds to propagate over greater distances compared to air⁴. Estimating the detection ranges between a particular sound-producing species and a receiver can give crucial information about its spatial distribution in an ecosystem. These calculations rely on the measurements of the source level (SL, *i.e.* the sound pressure level recorded at 1 m from the source) and the transmission loss (TL, *i.e.* the attenuation of the sound as it propagates away from the source) of animal sounds underwater. For example, marine mammal sounds can be detected kilometres away in shallow and deep oceans with hydrophones^{5–7}. Fish also produce sounds in shallow waters that can be detectable from few meters^{8,9} to hundreds of meters away^{10,11}. However, data available on sound propagation and detection ranges for crustaceans are scarce, though crustaceans are known to emit a large diversity of sounds^{12–14}.

Marine arthropods produce sounds that are mostly characterized by broadband pulses, *i.e.* short transient sounds with a large bandwidth^{15–17}. Estimating their sound propagation may be challenging as they inhabit shallow coastal waters (at depths below tens of meters). This implies complex sound fields due to physical constraints such as the presence of boundaries created by the water surface and the seabed^{18,19}, and it is thus difficult to accurately model sound propagation²⁰. Until now, detection ranges of crustacean sounds have relied on crude estimations of SLs performed using distant measurements that are then artificially back-propagated to 1 m by using theoretical propagation models^{21,22}. In addition, some studies have been performed in tanks. Tank experiments are very convenient since distances between receivers and animals can be precisely measured²³. However, tank acoustics are complicated. The relatively small volumes and close boundaries of tanks highly affect sound propagation as well as SL estimates^{13,14}; these tank effects have been largely ignored by most previous studies (as a counter example, see¹⁵ for an experimental illustration on the differences of crustacean sounds recorded in tanks and *in situ*). Thus, there is a need to combine theory with empirical measurements of site-specific sound propagation

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to obtain reliable SL and TL predictions for estimating detection ranges of crustacean sounds. For this purpose, arrays of hydrophones are useful because they can accurately estimate SL and TL in shallow waters^{11,24}.

Estimating SL requires an accurate knowledge of the distances between sound producers and receivers. This problem is likely easier for many benthic crustaceans than for marine mammals and fish, since they have a relatively low mobility. Spiny lobsters are particularly good models for performing such studies because they produce specific sounds termed “antennal rasps”. While these antennal rasps are characterized by trains of broadband pulses with sound intensity spread over a wide bandwidth, their spectrums are dominated by low frequencies (i.e. below 1 kHz^{15,25}). In addition, it is possible to induce sound production by handling the animals underwater (which aims to imitate a predator attacking^{15,16,25}). Hence, these animals are practical for accurately measuring SL and propagation of produced sounds over different distances, while precisely controlling their positions. Interestingly, sounds produced by spiny lobsters have similarities with insects²⁶. Several studies in terrestrial arthropods have shown that the intensity of their sounds depends on body size^{27,28}. If such a relation also exists in spiny lobsters, this implies that larger individuals likely produce higher amplitude sounds compared to smaller animals. As a result, these large individuals should be detectable over longer distances. In addition, a recent study¹⁵ recorded antennal rasps from the European spiny lobster (*Palinurus elephas*) underwater and reported high peak-to-peak sound pressure levels (SPL) up to 170 dB re 1 μPa^2 at 20 cm from the animals. Such elevated SPLs imply these sounds could be detectable during *in situ* PAM studies, which is needed to better manage this highly commercially valuable and vulnerable species that has become scarce in European coastal waters due to overfishing^{29,30}. However, it is now crucial to understand the variability of their sounds (i.e. with animal size), how they propagate and at what distances they can be detectable underwater with hydrophones before PAM can be used operationally to monitor spiny lobsters.

In this context, the aim of this study was to provide new insights on the propagation features of broadband sounds produced by a marine crustacean, the European spiny lobster (*P. elephas*), in a shallow coastal water area. First, we measured the SPLs of individuals from various sizes (from 2.6 to 13.5 cm of carapace length) using a linear array of 8 hydrophones placed between 0.5 and 100 m from the animals. Using this set-up, the SLs, in terms of SPLs, were obtained at 1 m from the animals. Secondly, we estimated the associated TLs using a simple model $a \times \log_{10}(r)$, with r being the source-receiver distance and a being the model parameter to be calculated, and we compared the estimated TLs with theoretical models. Based on these results, the detection ranges (and their variability with animal size) were estimated using different conditions of ambient noise levels (ANL) and TL models. Lastly, we examined changes in the spectral contents of the antennal rasps with increasing distance from the spiny lobsters, and discussed their potential ecological implications.

Results

During the experiment, the wind state ranged between 0 (calm) and 2 (light breeze) on the Beaufort scale, corresponding to speeds between 1 and 6 knots. Seawater temperature was 15.3 °C and salinity was 35. The water depth was 9 m. Anthropogenic noise from a near marina contributed to the low frequencies (below 1 kHz) in the recorded ambient noise, and was mainly produced by ship motor noise and the chains of the boats’ buoys rubbing against the bottom. However, the ANL in the frequency band from 10–78 kHz was quieter compared to the ANL calculated over the entire (0.001–78 kHz) frequency band (over 20 dB difference), and varied slightly across the different hydrophones with a mean of 88 ± 4 dB re 1 μPa^2 . The sources of ambient noise in the frequency band 10–78 kHz were attributed to isolated broadband pulses from unknown sources.

We extracted and analyzed manually a total of 1560 antennal rasps from the sound recordings of spiny lobsters ($N = 24$) with the linear array of 8 hydrophones placed between 0.5 and 100 m. This total number of analyzed antennal rasps corresponds to the 1920 antennal rasps that were recorded (10 per animal and per recording distance), minus 360 antennal rasps that were not analyzed because the signal-to-noise ratio was too low (i.e. the antennal rasps were buried in the ambient noise). Indeed, antennal rasps from intermediate ($N = 7$), small ($N = 3$) and very small ($N = 5$) individuals were not recorded beyond 50, 20 and 5 m, respectively (see Fig. 1). In marked contrast, all sounds from large spiny lobsters ($N = 9$) were recorded on all the hydrophones, with distance up to 100 m.

The SL_{pp} varied significantly and positively according to the body size of spiny lobsters (Pearson: $r = 0.910$, $t = -10.316$, $N = 24$, $df = 22$, $p < 0.001$). Indeed, we calculated a maximum difference of 35 dB between the smallest ($SL_{pp} = 132$ dB re 1 μPa^2 ; $CL = 2.6$ cm) and the largest (167 dB re 1 μPa^2 ; $CL = 13.5$ cm) spiny lobsters (Fig. 1). The maximum calculated SPL_{pp} was 172 dB re 1 μPa^2 and was produced by the largest individual ($CL = 13.5$ cm) at 0.5 m.

The TL models estimated as $a \times \log_{10}(r)$ from the dataset of SPL_{rms} vs. distance did not significantly vary with body size (Pearson: $r = -0.175$, $t = -0.733$, $N = 19$, $df = 17$, $p = 0.474$), as expected. The estimated TL parameter a ranged between 16.1 and 19.5 (Fig. 2). By fitting the results amongst all individuals (except the very small ones), the global TL parameter a was 17.6 which is between the theoretical models of practical ($a = 15$) and spherical ($a = 20$) TLs (Fig. 2).

Detection ranges of the antennal rasps produced by all spiny lobsters were estimated per group of body size by considering the calculated SL_{rms} and global TL ($a = 17.6$). Their values are summarized in Table 1. Under the ANL conditions encountered during the experiment, we estimated that large individuals can be recorded up to 250 m, with the largest individual ($CL = 13.5$ cm) being detectable up to 410 m (Table 1). Interestingly, the estimated detection ranges for smaller individuals are less than 100 m, and are thus covered by the range of our array. Thus, these estimated values are consistent with the values observed on the *in situ* recordings. Indeed, intermediate, small and very small spiny lobsters could not be recorded at 100, 50 and 10 m (respectively; see Fig. 1), which matches our estimations of detection ranges (Table 1). In a theoretical low (but still realistic) ANL, large individuals can be detectable at 750 m, with the largest individuals ($CL = 13.5$ cm) being recorded up to 1080 m. By considering a practical loss model for TL (the most realistic), large spiny lobsters could be detectable at the

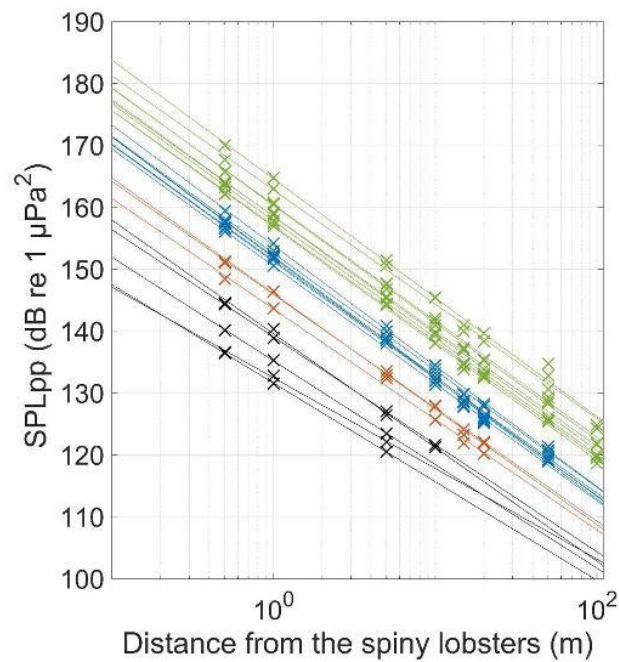


Figure 1. Evolution of sound pressure levels in peak-to-peak (SPL_{pp} ; crosses) recorded at different distances from the spiny lobsters (between 0.5 and 100 m) and their calculated fit curves using logarithmic models (continuous lines). Each point represents the mean SPL_{pp} averaged on 10 sounds. The x-axis is in logarithmic scale. Each value calculated at 1 m corresponds to the source level (SL_{pp}) of the spiny lobsters. The colors are related to the body size of the spiny lobsters (green: large, blue: intermediate, orange: small, black = very small).

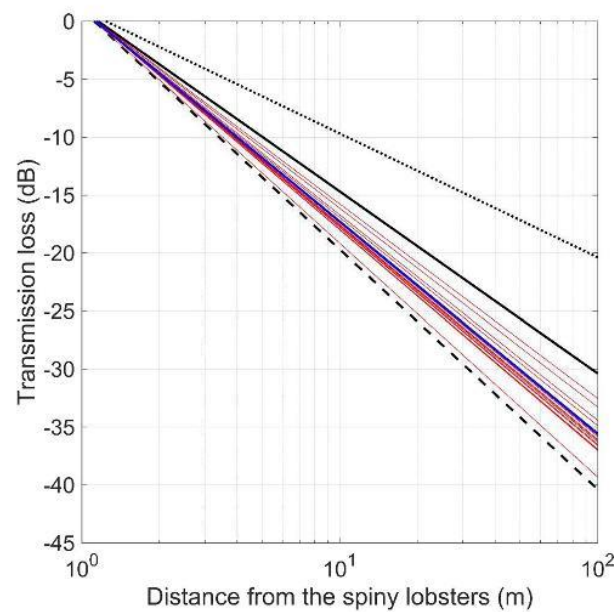


Figure 2. Transmission losses (TL) estimated for 19 spiny lobsters (red lines) based on the dataset SPL_{rms} vs. distance, and the global fitted TL (blue line, $a = 17.6$). Black lines represent theoretical models of TL: dotted is cylindrical ($a = 10$), continuous is practical ($a = 15$), dashed is spherical ($a = 20$).

kilometre scale under the theoretical ANL, with largest individuals being detected up to 1740 m (Table 1). Using an attenuated cylindrical loss model for TL (the least conservative), all spiny lobsters (except the very small ones) may be detectable at the kilometre scale under the theoretical low ANL, with largest spiny lobsters being detected up to 3610 m (Table 1).

	TL	Wenz 5 knots		This study	
		Min	Max	Min	Max
		ANL = 81	ANL = 86	ANL = 93	ANL = 98
Large (N = 9)	17.6	750	500	250	150
	15	1330	940	510	300
	10	3040	2450	1690	1180
Intermediate (N = 7)	17.6	390	230	100	60
	15	850	550	270	140
	10	2250	1710	1030	620
Small (N = 3)	17.6	210	120	50	27
	15	420	240	100	50
	10	1510	1030	500	240
Very small (N = 5)	17.6	70	40	15	8
	15	130	70	25	10
	10	620	330	100	40

Table 1. Estimations of detection ranges (in m) of antennal rasps produced by spiny lobsters underwater. The averaged values are reported per group of size-matched spiny lobsters (see Material and Methods for details). The different transmission losses (TL) used correspond to the global TL ($a = 17.6$) calculated between all spiny lobsters (except the very small ones), and the theoretical models of cylindrical TL ($a = 10$) and practical ($a = 15$) TLs. Min is signal-to-noise ratio (SNR) = 5 dB and max is SNR = 10 dB.

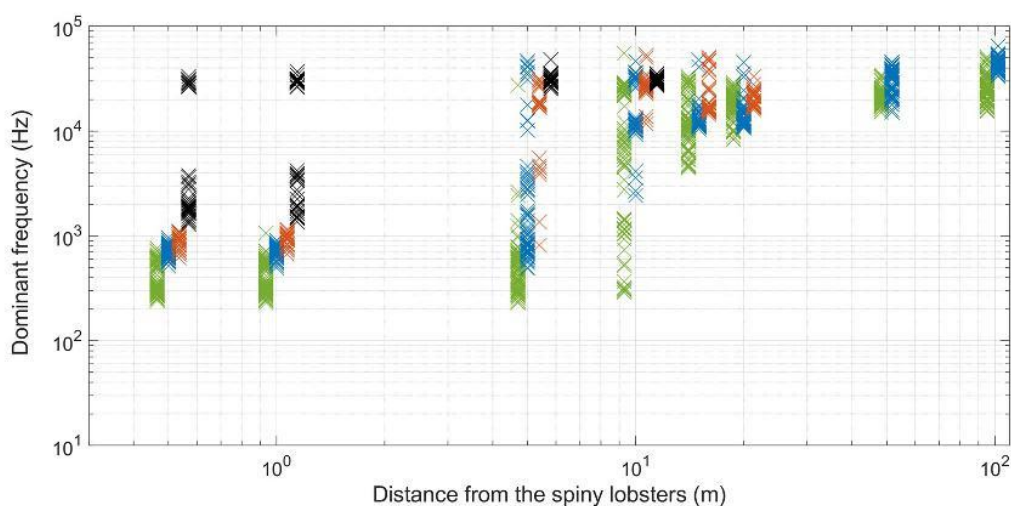


Figure 3. Dominant frequencies calculated on the recorded antennal rasps as a function of the animal-hydrophone distance. Each point represents the value from one antennal rasp. The colors are related to the body size of the spiny lobsters (green: large, blue: intermediate, orange: small, black: very small). Both x- and y-axis are in logarithmic scale.

Overall, a clear pattern occurred in changes of the spectral content of recorded antennal rasps with increasing distance. The spectral content was dominated by low frequencies (<1 kHz) close to the spiny lobsters (<10 m) whereas only high frequencies (>10 kHz) remained far from the animals (>10 m; Figs. 3 and 4). However, antennal rasps produced by very small spiny lobsters did not present any low frequency content (<1 kHz) even at 0.5 m (range 1.3 to 31.3 kHz). The low frequency content was probably masked by the ambient noise due to their low intensity features. Above 10 m, all recorded antennal rasps had dominant frequencies only between 10 and 60 kHz (Figs. 3 and 4).

Discussion

Passive acoustic monitoring. In the bioacoustic literature, only one study used passive acoustics to monitor spiny lobsters (*Panulirus japonicus*) underwater³¹. The authors found an increase in the production of antennal rasps on the nights of large tidal changes, which complement the ecological knowledge on the nocturnal behaviour of spiny lobsters³². However, the propagation features of the recorded antennal rasps were not assessed, and thus the detection ranges at which spiny lobsters produced sounds were not quantified, which drastically limits the impacts of the corresponding study. To fill this gap, the present paper is the first to experimentally examine

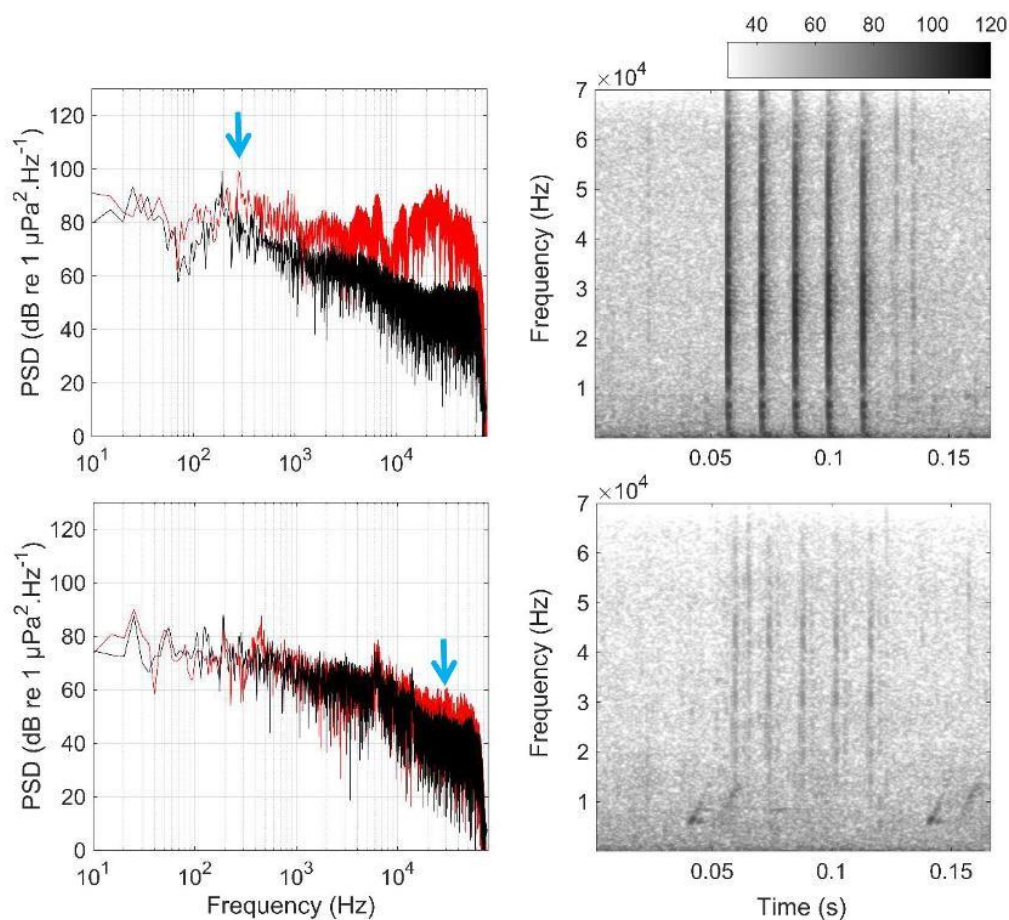


Figure 4. Power spectral densities (PSD, left) and spectrograms (right) of an antennal rasp produced by a large spiny lobster (carapace length = 13 cm) at 1 m (top) and 100 m (bottom). The black lines are the ambient noise recorded at the same distances. The blue arrows show the dominant frequencies of the recorded antennal rasp, calculated at 0.3 kHz at 1 m while it was calculated at 30 kHz at 100 m. The color scale bar is in dB re $1 \mu\text{Pa}^2 \cdot \text{Hz}^{-1}$.

the propagation features of the antennal rasps produced by the European spiny lobster (*P. elephas*) in shallow waters using a linear array of hydrophones with a range of 100 m.

Although SL_{pp} values have been reported for several marine species of mammals and fish, this is the first time that SL_{pp} values for a marine crustacean are reported from direct *in situ* recordings. Spiny lobsters produce loud antennal rasps, with SL_{pp} up to 167 dB re $1 \mu\text{Pa}^2$ for the largest individuals (CL = 13.5 cm; Fig. 1). Overall, our results confirm the range of values obtained by a recent study¹⁵ with the same species where SPL_{pp} (calculated only at 20 cm from the source) were calculated above 170 dB re $1 \mu\text{Pa}^2$. The antennal rasps produced by spiny lobsters have exceptionally elevated SL_{pp} among crustaceans. Indeed, only snapping shrimps have been reported to produce higher SL_{pp} ^{12,17}, estimated in tanks up to 215 dB re $1 \mu\text{Pa}^2$. We also found that SL_{pp} of spiny lobsters vary significantly and positively with their body sizes, with larger individuals producing higher amplitude sounds compared to smaller individuals. Indeed, the smallest individuals (CL = 2.6 cm) had SL_{pp} calculated at 132 dB re $1 \mu\text{Pa}^2$, which was 35 dB less compared to the largest individuals (CL = 13.5 cm; Fig. 1). These variations in sound intensity with body size have already been described in insects whose sound production mechanisms are similar to spiny lobsters^{26–28}. To our knowledge, no studies have examined ontogenetic variations of sound intensity in crustaceans. Further studies should relate these variations with the development of sound-producing structures in spiny lobsters.

The use of linear arrays of hydrophones is known to be useful to estimate SL and TL for marine mammals and fish^{11,24}. In marked contrast to these highly mobile animals, spiny lobsters produce sounds while handling, which enables precise control of their distances from the recording hydrophones. This allowed us to perform accurate measurements of the SPL_{pp} and SPL_{rms} over distance from the spiny lobsters (between 0.5 and 100 m; Fig. 1). While we found that SL_{pp} (and SL_{rms}) varied with body size, we did not find any significant relationship between the different coefficients of TL and the body sizes of the spiny lobsters. This result was to be expected: the physics of sound propagation does not depend on the sound source, and it is thus independent from the animal body size. The estimated TL [$17.6 \times \log_{10}(r)$] is consistent with sound propagation in shallow water^{18–20}, but different

from simplistic models (e.g. cylindrical losses) that are often used in bioacoustics. This clearly demonstrates the importance of correctly assessing sound propagation in shallow waters to study crustaceans' sounds. Last but not least, we only recorded sounds produced by spiny lobsters while facing the linear array of hydrophones (i.e. on the same axis). Thus, we did not quantify the potential directivity of their sound source. The use of three-dimension arrays will be useful in further studies to better describe the directivity and 3D propagation of antennal rasps in shallow waters¹¹.

An important application of measuring accurately SL and TL is the estimation of detection ranges at which animals can be detectable underwater with hydrophones. We found that the SLs of spiny lobsters varied with body size, and thus their detection ranges too. Indeed, during the sound recordings, only large individuals were recorded with the hydrophone placed at 100 m (Fig. 1). In marked contrast, intermediate, small and very small individuals were not recorded with our hydrophones above 50, 20 and 10 m, respectively (Fig. 1). These results were confirmed through the detection ranges estimated using the passive sonar equation, where intermediate, small and very small individuals can be detectable up to 100, 50 and 15 m, respectively (see Table 1). Under the ANL recorded during the experiment, large individuals are expected to be recorded at 250 m underwater with the largest spiny lobsters (CL = 13.5 cm) being detectable above 400 m (Table 1). Using a theoretical low (but still realistic) ANL³³, the detection ranges for large individuals were estimated at 750 m with the largest individuals being detectable up to 1080 m. In this study, the global TL calculated for spiny lobsters ranged between the theoretical models of practical and spherical TL, which are the most conservative models of TL (i.e. loss of 40 dB at 100 m for the spherical TL). By considering the least conservative model of TL (cylindrical), large spiny lobsters may be detectable at the kilometre scale under the theoretical ANL, with largest individuals being detected up to 3000 m (Table 1). This is consistent with a previous study which recorded broadband sounds likely produced by unknown species of invertebrates at kilometres away from coral reefs³⁴. This result is particularly important, because it demonstrates the possibility to use PAM for studying spiny lobsters underwater. Such studies would have a large spatial resolution, which is highly valuable for assessing their absence–presence and activity patterns in their environments, as shown for marine mammals and fish^{35,36}.

Ecological relevance. From an ecological point of view, the detection ranges discussed above may not be relevant for spiny lobsters. A more interesting quantity is the communication distance^{9,11}, i.e. the distance over which animals can “hear” each other. Communication distances are more difficult to assess because it requires additional information about the animal hearing abilities. To our knowledge, no audiogram has been performed in spiny lobsters, and nothing is known about their exact hearing sensitivity. However, it is thought that most crustaceans are sensitive to low frequency particle motion (i.e. below 1 kHz)^{37,38}. Assuming that this hypothesis holds true for spiny lobsters, our study may be used to roughly estimate the communication distances by evaluating the detection ranges of the low frequencies from antennal rasps. Nonetheless, we emphasize that only a rough estimate can be obtained since (1) we measured only sound pressure, and (2) the hearing capacity of spiny lobsters is not known. The following discussion will thus be qualitative, rather than quantitative.

The spectral content of the recorded antennal rasps was different according to the distance from the spiny lobsters. Indeed, while low frequencies (<1 kHz) dominated close to the sound source (<10 m), only high frequencies (>10 kHz) remained at higher distances (>10 m; Figs. 3 and 4). Since the spiny lobsters may be sensitive to low frequencies only (<1 kHz), the long-distance high-frequency antennal rasps are likely out of the hearing range of the animals. Thus, we can conclude that the communication distances are much shorter than the detection ranges. On the specific dataset studied here, assuming that the animals are sensitive to frequencies below 1 kHz, the communication distances would be no more than 10 m (see Fig. 3), which is consistent with what is known for fish^{8,9,11}.

The apparent frequency shift of the antennal rasps is due to sound propagation and ambient noise, and not ecological reasons. Indeed, the ANL in the low frequencies (below 1 kHz) was 20 dB louder compared to the higher frequencies (above 10 kHz). As sounds propagate away from the source, their intensities decrease. Thus, at large ranges (above 10 m), the apparent dominant frequencies (<1 kHz) of the antennal rasps are fully masked by the high-power low-frequency ambient noise. The recorded sounds have therefore dominant frequencies only above 10 kHz, i.e. in the frequency band where the high frequency part of the antennal rasps is not masked by the ambient noise (see Fig. 4). Interestingly, another study³¹ recorded sounds produced by *Panulirus japonicus* and the authors found that the recorded antennal rasps were also dominated by high frequencies (above 10 kHz). This study was performed in a shallow water area at depths similar as our study (between 7 and 11 m). Thus, our results show that the apparent high dominant frequencies observed at large ranges are due to acoustic masking, and likely have no ecological meanings for spiny lobsters.

In this study, we recorded sounds in very shallow waters mainly for practical reasons. However, *P. elephas* is known to inhabit coastal waters from the shore up to depths of 200 m, and large individuals are most commonly found between 50 and 100 m depths^{39,40}. In addition, we recorded antennal rasps in a flat sandy bottom, while spiny lobsters tend to live in rocky habitats^{29,41}. Overall, detection ranges and communication distances may change depending on the environment both because of sound propagation and ambient noise. We evaluated detection ranges by using different propagation models, and the obtained results ranged between 10 and 3000 m (depending on the size of the animals). Although simple, the models considered here represent reasonable bounds within which more realistic models may predict propagation. It is thus expected that the order of magnitude of the detection ranges evaluated in this paper hold also for other environments. On the other hand, the communication distances have been evaluated without using propagation models. Nonetheless, the estimated values are of the same range as the water depth (i.e. 10 m). Their propagations are thus impacted little by the environment (TLs are usually modeled using simple spherical losses)⁴². Hence, we predict that the order of magnitude of the communication distances would be similar in other environments. This must now be confirmed

Hydrophone			Recorder			Distance from the spiny lobsters (m)
Model	Flat frequency response (kHz)	Sensitivity (dB re 1 V μPa^{-1})	Model	Gain (dB)	Fs (kHz)	
HTI-99-HF	0.002–125	–174.9	EA-SDA14 (RTSys, France)	0	156	0.5
		–174.7		0	156	1
		–174.9		15	156	5
		–175		15	156	10
		–174.8	EA-SDA14 (RTSys, France)	15	156	15
HTI-96-MIN	0.002–30	–163.8	Wildlife Acoustics Song Meter (Model SM2)	24	96	50
		–174.7		15	156	20
HTI-92-WB	0.002–50	–155.5	EA-SDA14 (RTSys, France)	15	156	100

Table 2. Characteristics of the recording devices used for the linear array of hydrophones (placed between 0.5 and 100 m from the spiny lobsters) during *in situ* recordings. Fs: frequency sampling.

through the development of audiograms and the measurements of particle motion generated by the antennal rasps underwater^{37,43}.

Towards a better monitoring of a vulnerable and cryptic species. In conclusion, the use of a linear array of hydrophones permitted us to examine for the first time the propagation features of the antennal rasps produced by spiny lobsters in a shallow water area. The accurate measurements of SL and TL allowed us to estimate detection ranges underwater by considering different conditions of ANL. We notably found that sounds from spiny lobsters can be detectable kilometres away underwater with hydrophones. These results will be helpful for further PAM studies because they can allow potential detection, localization and even estimation of densities of *P. elephas* over large spatial and temporal scales underwater^{44,45}. The development of such non-invasive and non-destructive tools is needed to better manage this highly commercially valuable and vulnerable species that has become scarce in European coastal waters due to overfishing^{29,30}.

Methods

Animal collection, characteristics and care. For the experiments, we used a total of 24 *P. elephas* individuals of a wide range of sizes. Only inter-moult individuals with full sets of intact appendages were selected for this study.

We carefully collected 17 juveniles by hand while scuba diving in the Bay of Perros Guirec (48°50'2.044" N, 3°26'28.312" W) at depths between 10 and 25 ms during two diving sessions on May 28th, 2019. Juvenile individuals (3 males and 14 females) had carapace lengths (CLs) between 2.6 and 8 cm, as measured from the anterior tip of the rostrum to the medial point of the posterior carapace margin. Large adult individuals were bought from local fishermen several days after they were captured in the Iroise Sea on May 21st, 2019. These 7 large spiny lobsters (2 males and 5 females) had CLs between 9.5 and 13.5 cm.

After capture, all individuals were immediately transferred to an isolated, quiet room in the facilities of the Institut Universitaire Européen de la Mer (IUEM) in Plouzané (France). They were placed in holding tanks of different dimensions according to their size: three plastic-sided rectangular tanks (0.60 m × 0.50 m × 0.35 m, length × width × effective height; seawater volume = 0.1 m³) and two plastic-sided square tanks (1.0 m × 1.0 m × 0.6 m, 0.6 m³). There were 4 to 8 individuals per holding tank. Before they were placed in these tanks, all individuals were tagged using alternating small white and black rubber bands placed on the base of their second antennae. Each tagged individual was described (size, sex) and given an identification number.

Holding tanks were continuously supplied with the same sand-filtered seawater pumped from the Bay of Brest. During the holding period, temperature was 14.6 ± 0.6 °C and salinity was 34.7 ± 0.1. All animals were fed with fresh pieces of fish (mackerel) *ad libitum*. They were kept under a 12:12 photoperiod; daylight conditions (from 08:00 am to 08:00 pm) were simulated with fluorescent light tubes placed above the holding tanks. Several sections of rigid, PVC pipes associated with large rocks were provided as shelters in each tank. Animals were acclimatized at least 15 days in these holding conditions before they were used in the at-sea recording experiment.

Sound recordings and video. A linear array of 8 hydrophones was used to record sounds produced by the spiny lobsters (see Table 2 for characteristics of the recording devices). The 8 hydrophones were spaced relative to the handled spiny lobsters at 0.5, 1, 5, 10, 15, 20, 50 and 100 m. The 2 closest hydrophones (0.5 and 1 m) were set with a gain of 0 dB which permitted us to characterize the powerful antennal rasps without clipping the recorded sounds (*i.e.* sound saturation).

Video recordings were made during *in situ* recordings using two GoPro HERO3 cameras at a recording rate of 29.97 frames per second. The videos allowed confirmation of sound production by each spiny lobster tested,

and also provided the time at which the handled individuals were placed at the source point during the sound recordings. They also validated the identification of each tested individual by checking the rubber bands on the spiny lobsters' second antennae.

Location and characteristics of the experimental site. The experimental site where sound recordings were performed was located in the Bay of Saint Anne de Portzic (48°21'32.951" N, 4°32'59.024" W) in the Bay of Brest, just beneath the facilities of the IUEM where spiny lobsters were held. It was located about 100 m outside a marina hosting 120 recreational boats. It is a shallow water area with depths varying between 15 m during high tide and 9 m during low tide. The bottom is flat and composed of homogenous, fine sand with empty shells.

Experimental set up. The day prior the recording experiment, while scuba diving, all spiny lobsters were transferred into three galvanized steel cages (1.0 m × 1.0 m × 0.5 m, 0.5 m³) placed side by side linearly on the bottom near a rocky dyke. Sections of rigid PVC pipes were provided as shelters. Spiny lobsters were acclimatized for 24 hours in these conditions to recover from transport and handling.

The next day (June 14th, 2019), while scuba diving, the linear array of hydrophones was built in front of the center holding cage. First, a rope was laid on the substrate, which was previously marked at each distance where the different hydrophones should be placed. Then, hydrophones were attached 0.5 m above the bottom to metal rods anchored with concrete tubes at each mark placed on the rope. Cables were anchored to the bottom with lead weights and recorders were laid on the bottom. Because the Wildlife recorder had a positive buoyancy, it was anchored to the bottom using a lead weight. Thus, its hydrophone (placed at 50 m from the spiny lobsters) was located at 1 m above the bottom. The two cameras (same model GoPro as mentioned above) were placed on the top of the 2 outside cages, in front of the center cage. Then, the boat transporting scuba divers was anchored 200 m away from the cages, and its motor was shut down. Sound recordings were performed during low tide to avoid tidal currents. Before the recording experiment started, the ambient noise was recorded for 10 minutes without scuba divers underwater. Next, each spiny lobster was gently picked up, handled one by one, and positioned at the source point. The source point, defined as the point where spiny lobsters were recorded, was located at the beginning of the rope, at 0.5 m from the first hydrophone. Each individual was maintained at the same distance above the bottom (0.5 m) as the hydrophones during recordings, and the spiny lobsters were held so that they faced the linear array of hydrophones. Thus, the body of the animals was on the same axis as the linear array of hydrophones. We chose to handle spiny lobsters to elicit their sound production, as this method is commonly described in the bioacoustic literature on spiny lobsters^{15,16,25}. Each sound recording for the different spiny lobsters lasted between 20 and 30 s. During each sound recording, the two scuba divers stopped their breath to avoid the emission of intrusive noise related to air bubbles. In total, the recording experiment lasted 60 min. In the end of the recordings, five sharp raps were made on the cage walls which permitted us to synchronize both hydrophones and GoPros.

Sound analysis. *Sound features of antennal rasps.* Synchronized recordings of sounds (in.wav format) and videos were first analyzed to confirm sound production by each tagged spiny lobster. Then, each antennal rasp was extracted manually using the Audacity software (version 2.1.1⁴⁶). Antennal rasps were defined as pulse trains composed of at least several pulses separated by less than 20 ms from each other. Hence, any isolated pulses present in the recordings were not analyzed here. We performed sound analysis on a total of 10 antennal rasps per spiny lobster and per distance (80 sounds analyzed per individual in total). The same sounds were analyzed at the 8 different distances for each spiny lobster. All sequences were then processed using custom MATLAB scripts (version 9.1; The MathWorks).

We calculated the intensity features of the antennal rasps based on their sound pressure levels (SPL, in dB re 1 μPa²) both in peak-to-peak (for biological interpretation) and root-mean-square (for detection range estimations using the passive sonar equation).

As these sounds are pulse trains characterized by short and transient pulses, we first chose to calculate the peak-to-peak SPL (SPL_{pp}) which is the most representative and practical intensity feature for these types of biological sounds^{15,47}. As we recorded antennal rasps at several distances from the spiny lobsters, the SPL calculated at 1 m was referred to as the source level (SL_{pp}). When pulse trains were affected by low frequencies related to ambient noise (below 50 Hz), especially at long distances (50 and 100 m), we measured the SPL_{pp} based on the pulse with the highest and lowest amplitude of the train to avoid overestimating their values. When pulse trains could not be isolated from the ambient noise, we did not calculate their SPL_{pp}. We then averaged the SPL_{pp} calculated per distance and per individual for further analysis. The SPL_{pp} and SL_{pp} were used for biological sound characterization. Because these values varied according to the body size of the spiny lobsters (see Results), we chose to regroup the averaged values per group of size-matched individuals for a better overall description. Four different groups of body sizes were defined and termed as follow: large (8.0 < CL < 13.5 cm, N = 9), intermediate (6.4 < CL < 7.3 cm, N = 7), small (4.2 < CL < 4.8 cm, N = 3) and very small (2.6 < CL < 3.1 cm, N = 5).

We also calculated the SPL and SL as root-mean-square (SPL_{rms} and SL_{rms}, respectively) by integrating the power spectral density (PSD, in dB re 1 μPa².Hz⁻¹) of the antennal rasps between 10 and 78 kHz (bandwidth where intensity from the antennal rasps remained above 10 m from the spiny lobsters, see Results). The SPL_{rms} at 50 m was calculated over the 10–48 kHz frequency band, because the system specification did not allow measurements of frequencies above 48 kHz (see Table 2). Because antennal rasps are characterized by pulse trains, the SPL_{rms} and SL_{rms} values averaged on the entire length of the antennal rasps would be underestimated. We instead chose to calculate them on each pulse inside the pulse trains over a 1 ms window length (Fast-Fourier Transform size: 156 points) centered on the pulse. Then, each value was averaged over all pulses present in an antennal rasp to obtain its mean SPL_{rms} and SL_{rms}. As for SPL_{pp} and SL_{pp}, the SPL_{rms} and SL_{rms} values were averaged on 10

antennal rasps per individual. The SPL_{rms} and SL_{rms} values were used to estimate transmission loss (TL), as well as to compute the passive sonar equation to estimate detection ranges⁴².

We also calculated the dominant frequency (in kHz) of each antennal rasp, represented as the frequency where the PSD was maximal. At large distances, some sounds were lost in the ambient noise while looking at the time domain signals, their SPL_{pp} and SPL_{rms} were thus not computed. However, they were still visible in the frequency domain; in this case, their dominant frequencies were estimated.

Ambient noise characterization. Recordings of ambient noise (10 min each) from the 8 hydrophones were first visualized to ensure the absence of antennal rasps. Because anthropogenic noise affected the ambient noise recordings during the experiment, sound sequences were both cut into 20 sequences of 30 seconds each, and we randomly selected 3 of 20 sequences from each recording. The sequences where anthropogenic noise (mainly shipping noise) was dominant were not taken into account in the analysis. We calculated the SPL_{rms} of all selected 30 s long sequences. This SPL_{rms} was calculated over the same frequency band as the antennal rasp SPL_{rms} and SL_{rms} (10–78 kHz), except for data from the Wildlife recorder, for which SPL_{rms} was calculated over the 10–48 kHz frequency band. This provided a mean value for the ambient noise at each hydrophone placement, and was referred to the ambient noise level (ANL).

Evaluation of transmission losses. The datasets of the averaged SPL_{rms} for each individual and for each distance were fitted with nonlinear least-squares regressions using custom-made scripts in MATLAB. We used the following equation⁴²:

$$SPL_{rms} = SL_{rms} - TL \quad (1)$$

where TL is the transmission loss (in dB). TL represents the loss of intensity due to the geometrical spreading of sounds in a physical medium⁴², and was calculated as the slope of the logarithmic regression between SPL_{rms} and the distance from the spiny lobsters, which was expressed as:

$$TL = a \times \log_{10}(r) \quad (2)$$

where r is the distance between the spiny lobsters and the hydrophones (in meters), and a is the geometrical TL term.

We obtained 19 different TL models using this method on the dataset generated by each animal (i.e. known SPL_{rms} , SL_{rms} and r). The measurements from the 5 very small individuals were not included in this analysis because we did not have enough measurement points as they were only detectable between up to 5 and 10 m (see Results). Moreover, a global TL coefficient was also estimated using a global dataset obtained by merging the sounds from the 19 spiny lobsters. We compared this global TL with other theoretical models of TL commonly used in the bioacoustic literature¹⁹. In theory, the spherical spreading loss ($TL = 20 \times \log_{10}(r)$) prevails near the source where sound propagates uniformly in all directions. The cylindrical spreading loss ($TL = 10 \times \log_{10}(r)$), prevails in shallow waters where sound cannot propagate as a spherical wave in all directions but only as a cylindrical wave bounded by the sea floor and the sea surface. We also used a combined TL, termed the ‘practical’ spreading loss ($TL = 15 \times \log_{10}(r)$), which occurs between the prediction of the two other spreading models described above⁴⁸.

Estimations of detection ranges. For the purpose of this study, we assumed that signal detection by hydrophones was primarily limited by the TL (previously calculated), the ANL and the absorption (α) of the high frequencies considering the detection ranges (i.e. kilometre scale).

Using the previous results, we estimated the detection ranges of the antennal rasps by resolving the passive sonar equation (in the frequency domain) for all 24 spiny lobsters⁴²:

$$SL_{rms} - TL - \alpha - ANL = SNR \quad (3)$$

Where:

- SL_{rms} is the source level in dB re $1 \mu Pa^2$ (in root-mean-square; averaged on 10 measurements per spiny lobster), calculated in the 10–78 kHz frequency band⁴².
- TL is the global coefficient of TL previously calculated for the 19 largest spiny lobsters. We also used the models of cylindrical and practical TL detailed above.
- α is the coefficient of attenuation, depending on the frequency of the sound. Here, it was used at the dominant frequency that was commonly found above 10 m in the recorded antennal rasps, which was estimated at 30 kHz (see Results). Thus, the coefficient of absorption was calculated to be 7 dB per km⁴⁹.
- ANL is the ambient noise level in dB re $1 \mu Pa^2$ (in root-mean-square) calculated over the same band of frequencies than the SL_{rms} (10–78 kHz). We used two different values of ANL. First, we reported the mean ANL recorded by our hydrophones during the study. Second, we used one theoretical (but still realistic) value of ANL based on Wenz curves and calculated with a wind speed of 5 knots in the same frequency band than the ANL *in situ*³³.
- SNR is the signal-to-noise ratio which corresponds to the minimum threshold needed for the hydrophones to detect the sound above the ANL. We used two different SNRs of 5 and 10 dB widely accepted for sonar systems⁴².

Because our sound recordings were performed in shallow waters (<10 m), we considered the water column as non-stratified. Thus, the effects of sound speed were assumed to be independent of depth⁴, and were not taken into account in this equation. Also note that in theory, the absorption coefficient α should be embedded into our estimated TL model. However, the impact of α is relatively small over our array range, with a loss smaller than 1 dB, which is negligible with respect to the geometrical TL. On the other hand, at larger ranges, the impact of α becomes important. We thus decided to add α in Eq. (3); this ensures that detection ranges are not over estimated.

Statistical analysis. We examined the correlations between SL_{pp} and TL with body size using Pearson tests ($\alpha = 0.05$). Analysis were performed using R version 3.5.1⁵⁰.

Ethical statement. Experiments with European spiny lobsters are not subject to restriction for animal scientific research according to the French legislation and the European Community Council Directive of September 2010 (2010/63/UE). However, we followed the ARRIVE guidelines⁵¹ to ensure that all experiments were performed under good conditions. Animals were handled with care during the study and their health status were checked daily by the authors. No specimens were harmed during this study and there was no mortality. At the end of the study, 7 adults were kept in the laboratory for other experiments. All the other animals were released back into the environment where they were collected.

Data availability

The codes developed in Matlab for signal processing can be provided if requested by the reviewers. We can also communicate .wav files of antennal rasps recorded during the *in situ* experiment.

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Author contributions

Y.J., J.B. and L.C. designed the experiment, wrote and reviewed the manuscript. Y.J. and L.C. collected the data. Y.J. and J.B. carried out the analyses.

Competing interests

The authors declare no competing interests.

Additional information

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Titre : Ecologie acoustique du homard Européen (*Homarus gammarus*) et de la langouste rouge (*Palinurus elephas*)

Mots clés : Crustacés | Production sonore | Acoustique passive | Communication | Propagation

Résumé : Le rôle écologique des sons chez les crustacés est mal défini comparé aux mammifères marins et aux poissons. Or, comprendre l'importance des sons pour la biologie d'une espèce est crucial lorsque les impacts des bruits anthropiques sont recherchés. Par ailleurs, l'écologie cherche encore à développer de nouveaux outils de suivi par acoustique passive (PAM). L'enjeu principal de cette thèse était d'étudier la bioacoustique de deux espèces de crustacés à fort intérêt commercial et patrimonial en Europe : le homard Européen *Homarus gammarus*, et la langouste rouge *Palinurus elephas*. En tenant compte de l'effet physique des cuves sur les sons, nous avons mis en évidence la forte production de buzz entre homards mâles lors de rencontres agonistiques pour établir des statuts de dominance. Une approche neurophysiologique nous a ensuite permis de caractériser les capacités sensorielles des homards qui détectent les sons dans la même bande de fréquence que les buzz qu'ils émettent, renforçant l'hypothèse d'une communication acoustique. La deuxième partie de cette thèse présente comment, chez la langouste, leurs rasps d'antennes pourraient être utilisés avec du PAM par les biologistes et les halieutes. En effet, après avoir quantifié leur propagation in situ, nous avons mis en évidence que ces sons peuvent être détectés à l'échelle du kilomètre, et que leurs caractéristiques dépendent de la taille des individus. Nous avons également montré que ces rasps d'antennes sont largement énergétiques dans les basses fréquences (< 1 kHz), ce qui permet aussi de poser l'hypothèse de leur utilisation pour une communication acoustique. Les résultats de ce travail de thèse ouvrent des perspectives importantes sur l'impact potentiel des bruits anthropiques et le développement du PAM pour la gestion et conservation des crustacés.

Title : Acoustic ecology of the European lobster (*Homarus gammarus*) and the red spiny lobster (*Palinurus elephas*)

Keywords : Crustaceans | Sound production | Passive acoustics | Communication | Propagation

Abstract : The ecological role of the sounds produced by crustaceans is poorly known compared to marine mammals and fish. Understanding the importance of the sounds emitted by a species is critical to better apprehend the impacts of anthropogenic noise on their behaviours. In addition, marine ecologists are looking to develop new monitoring tool using passive acoustics (PAM). The aim of the PhD thesis was to study the bioacoustics of two crustacean species of high commercial and cultural interest in Europe: the European *Homarus gammarus*, and the spiny lobster *Palinurus elephas*. Taking into account the physical effects of tanks on sounds, we highlighted the high production of buzzing sounds between male European lobsters during agonistic encounters to establish dominance status. Further, we demonstrated using a neurophysiological approach that lobsters are able to detect sounds in the same frequency band than the buzzing sounds they produce, which strenghtens the hypothesis of an acoustic communication. The second part of this PhD thesis demonstrated the high acoustic potential of antennal rasps produced by spiny lobsters for PAM. Indeed, after quantifying their sound propagation in situ, we found that they can be detectable over kilometer scale and that their features depend on the size of the individuals. We also showed that these antennal rasps have a low frequency energetic content (< 1 kHz), which allowed us to state the hypothesis of their potential role for acoustic communication. This work raises new perspectives in marine ecology to study the impacts of anthropogenic noise and develop PAM tools for management and conservation measures of crustacean populations.