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A survey of NDT ultrasound transducers for sensing pre-moult tropical rock lobsters, *Panulirus ornatus*

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A R T I C L E I N F O

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A B S T R A C T

This study examined the novel application of non-destructive testing (NDT) ultrasound for detecting approaching moult in juvenile tropical rock lobsters (TRL), *Panulirus ornatus*. TRL juveniles are highly cannibalistic in culture, with up to 25% of lobsters lost to cannibalism at each moult. A pre-moult sensor could prove pivotal as moulting lobsters are the primary victim of cannibalistic predation and a reliable indication of pre-moult could trigger a suitable intervention measure. A series of structural changes take place beneath the exoskeleton in preparation for moulting, which leave little indication of pre-moult on the lobster's external surface. An opportunity exists for penetrative pre-moult detection via ultrasonic acoustic stimulation through the exoskeleton. NDT ultrasound is proposed as a possible pre-moult sensing technology. NDT ultrasound is comparatively low-cost, a requirement for replicated deployment to industry. This study explores the acoustic response from five NDT ultrasound transducers by scanning the dorsal carapace of juvenile TRLs.

A small diameter (13 mm) transducer with a 7 MHz centre frequency produced and measured a low amplitude, long duration signal segment in intermoult lobsters that was not present in pre-moult lobsters, forming a basis to distinguish intermoult from pre-moult lobsters. It is proposed that muscle detachment and growth of the new integument and a separating membrane disrupt sound conduction beneath the exoskeleton in a similar fashion to delaminated joint detection methods used in industry. These results show promise that a pre-moult sensor may be able to be based on NDT ultrasonic technologies and provide guidance for refining transducer specifications for further research.

1. Introduction

This study examined a novel application for non-destructive testing (NDT) ultrasound to detect approaching moult in juvenile tropical rock lobsters (TRL), *Panulirus ornatus*. Moulting is required for lobsters to grow, but it comes at the cost of being temporarily soft and vulnerable to predation, including from other lobsters [1–3]. Cannibalism during or shortly after moulting among juvenile TRLs in an aquaculture setting is a hurdle in the commercial production of lobsters. Kelly et al. [1] report loss of 20 to 25% per moult which translates into substantial cumulative losses over multiple moult cycles. Lobsters kept in isolation have comparatively reduced growth rates and increased stress [2–6]. Communal rearing maximises growth rates but introduces the challenge of safeguarding pre-moult lobsters until they have completed their moult.

A pivotal element missing in current strategies is a pre-moult sensor; a tool that could forewarn an imminent moult and potentially trigger an intervention. Ultrasound technology emerges as a promising penetrative pre-moult sensing solution as growing a new integument beneath the entire exoskeleton as an intrinsic requirement for moulting results in a changed surface, albeit concealed beneath the current exoskeleton. Further benefits of ultrasound include effective coupling in an aqueous medium allowing ultrasound scanning of lobsters without physical contact, and ultrasound technology operates efficiently without light, making it ideal for minimal disturbance of nocturnal lobsters in dark culture environments [3,7–9].

A comprehensive review of the literature found few prior published works on the subject of ultrasonic investigation of lobsters, or

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crustaceans, with all the works found using imaging ultrasound. Sutherland et al. [10] used ultrasonic imaging to visualize the growth of the new branchiostegite immediately beneath the exoskeleton of *P. ornatus*. Imaging ultrasound used for non-invasive studies of other crustaceans include, Haefner [11], who studied the heartrate of *Portunus gibbesii*, Gardner et al. [12] imaged the spermathecae of *Pseudocarcinus gigas* and Macrì et al. [13] investigated the cardiovascular and digestive physiology of *Procambarus clarkii*.

The expense of imaging ultrasound equipment is not conducive for repeated installation throughout a commercial lobster culture facility where cost is a key consideration in system design. With a long history of industrial applications, NDT transducers are varied, robust, reliable and relatively low cost when compared with advanced phased array and medical ultrasound. The use of NDT ultrasound on any crustacean, including lobsters, appears to have no peer, precedent or parallel in the literature. The crustacean exoskeleton is comprised of mineralised layers of chitin threads stacked with continuously changing orientation, resulting in helical stacking interspersed with pores [4,14,15]. The response of the exoskeleton to acoustic stimulation is undocumented and has no comparable material used in industry. An added complication exists because moulting of the exoskeleton in crustaceans is unique to the arthropods, further reducing the scope for meaningful comparisons with other research.

A consideration in favour of testing NDT ultrasound is that no quality or staging qualifications are required for sensor operation. This simplification reduces the complexity of pre-moult detection so that a signal segment that changes reliably prior to moulting will be sufficient. The binary operation of the sensor contrasts with the detailed moult staging system for crustaceans developed by Drach [16] and refined for *P. ornatus* by Turnbull [17]. This study uses the terminology of intermoult and pre-moult from the established staging system. 'Pre-moult' is used for juvenile lobsters on the day of their moult with fully developed ecdysial suture lines. 'Intermoult' is defined as one-week post-ecdysis extending to the earliest external visual signs of pre-moult as defined by Sutherland et al. [10] which include ventral surface pigment absorption and darkening of the region between the thorax and abdomen, the pleopods, and the uropods. The lobsters used in this study had a six-week moult period.

An experimental approach to begin accumulating knowledge and documenting acoustic properties of the exoskeleton has been used in this study. This exploratory study uses comparative analysis, including recovering, and rescanning the discarded post-moult exoskeleton (exuviae). With only water present inside the exuviae, rescanning the exuviae has the specific purpose to determine the delay time required to exclude the influence of the exoskeleton in scans from intermoult and pre-moult lobsters. By identifying and excluding the exoskeleton's effects from scans, scan features and delays attributable to ultrasonic interactions occurring within the intermoult and pre-moult lobsters can be determined.

Using five available transducers with centre frequencies of 1, 5, 7, 10 and 20 MHz, we aim to evaluate the interaction of acoustic properties of the lobster's exoskeleton with each of the five transducers for the purpose of guiding transducer selection for further targeted study. We aim to identify a signal segment, or segments, that distinguishes pre-moult lobsters from intermoult, and to interpret our findings using established acoustic mechanisms that can be related to structural changes occurring beneath the exoskeleton at pre-moult. We also aim to explore the necessary acoustic properties of the lobster's exoskeleton to aid in analysis and understanding of potential acoustic mechanisms for sensor operation. This three-tiered approach seeks to provide a foundation for future research and advancements in this emerging area of lobster aquaculture.

2. Test animals

2.1. Animal husbandry

The study was undertaken at the Institute for Marine and Antarctic Studies, (IMAS) University of Tasmania, Taroona over a duration of three months. Twelve hatchery-bred and reared juvenile TRLs, were utilised in this study. These lobsters had a carapace length and weight range of 35.9 – 50.4 mm and 46.9 – 123.1 g, respectively. The lobsters were of mixed sex with 5 females and 7 males in the population. The lobsters were maintained in a communal culture vessel (2.52 m $\times$ 0.96 m $\times$ 0.38 m – length $\times$ width $\times$ height; volume 920-L), on recirculating seawater, temperature 26.0 $\pm$ 0.5 °C, salinity 34.0 $\pm$ 0.8 ppt, pH 8.2 $\pm$ 0.1. Culture vessels were supplied with a continuous supply of water resulting in a turnover of 3 water exchanges per hour. The lobsters were fed twice daily with proprietary formulated pellets at a rate of 3.5% dry weight of animal body weight, adjusted to satiation. Cleaning of the tanks and removal of uneaten feed was done via siphon prior to each feeding event.

2.2. Moult stages for comparison

The moult stage groups for comparison were: 1). Intermoult lobsters, in this instance defined as one-week post-ecdysis extending to the earliest external signs of pre-moult; 2). Pre-moult, lobsters on the day of moulting with fully developed suture lines; 3). Exuviae, were recovered and scanned on the day following ecdysis. Care was taken to ensure the exuviae remained submerged at all times to prevent ingress of air bubbles into the exuviae.

2.3. Scan location

An anterior region between the base of the anterior dorsal large horns on the lobster's carapace was chosen for initial scanning. This region was used as the number of spines and pits is greatly reduced resulting in a flat, mostly uniform section of integument (Fig. 1).

3. Ultrasonic test equipment and configuration

3.1. Test equipment

The experimental system employed five transducers which are detailed in Table 1. Transducers S-7 and S-1 were contact transducers, with the other transducers being immersion type. All transducers were operated as immersion transducers using seawater for acoustic coupling. The S-7 transducer had a noted operational difference from its specification when operated in immersion, rather than in its designed surface contact role. All transducers were driven from an OpBox 2.1 ultrasound pulser/receiver and Optel OEM capture software for PC from Optel.

3.2. Setting the pulser/ receiver for each transducer

The Optel 20 MHz transducer (O-20) was supplied with the OpBox pulser receiver and the remaining transducers were 3rd party to this pulser receiver. Finding suitable settings on the OpBox for each transducer involved using combinations of transducers in transmit and receive mode so that incident pulses could be captured and compared. Pulse excitation power and duration were set to low values initially. The incident pulse was observed while the pulser excitement power and duration was adjusted. Excitement duration was found to have a strong effect on the incident pulse, with all transducers showing increased amplitude with increasing duration until a peak amplitude was reached, after which increased excitation duration had little effect. The exception being the S-10 transducer which started producing extended and distorted incident ultrasound pulses with extended excitation duration.

The excitation power was set at the half-way point (level 7 out of 15)

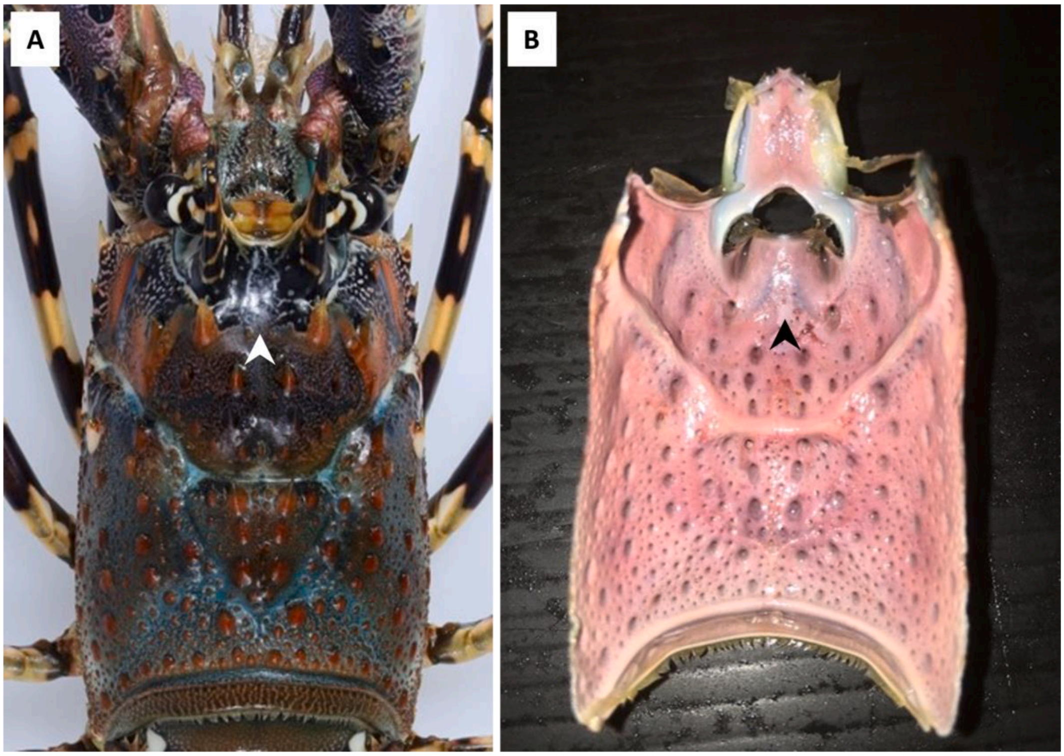

Fig. 1. Exterior and interior surfaces of the lobster exoskeleton of the dorsal carapace; A) exterior surface; and B) interior surface. Arrows indicate the region selected for scan sampling between the base of the large horns where the exoskeleton is approximately flat, with reduced spines and pits and almost uniform in thickness.

Table 1
Specifications for the five test transducers.

Key	Manufacturer	Country of Origin	Model	Centre Frequency (MHz)	Element Type	Element Diameter
O-20	Optel	Poland	17-009-04	20	Piezoceramic	¼ inch
S-10	Sensor Networks	USA	SensorScan 00-0113490 (1.6S Focus)	10	Piezoceramic	½ inch
S-7	Sensor Networks	USA	SensorScan 00-010603	7*	Piezoceramic	¼ inch
W-5	Waygate Technologies	Germany	22FO19RW	5	Piezocomposite	¾ inch
S-1	Sensor Networks	USA	SensorScan 00-010211MD	1	Piezoceramic	¾ inch

* Section 3.4 explains why this transducer is classed as 7 MHz in this study.

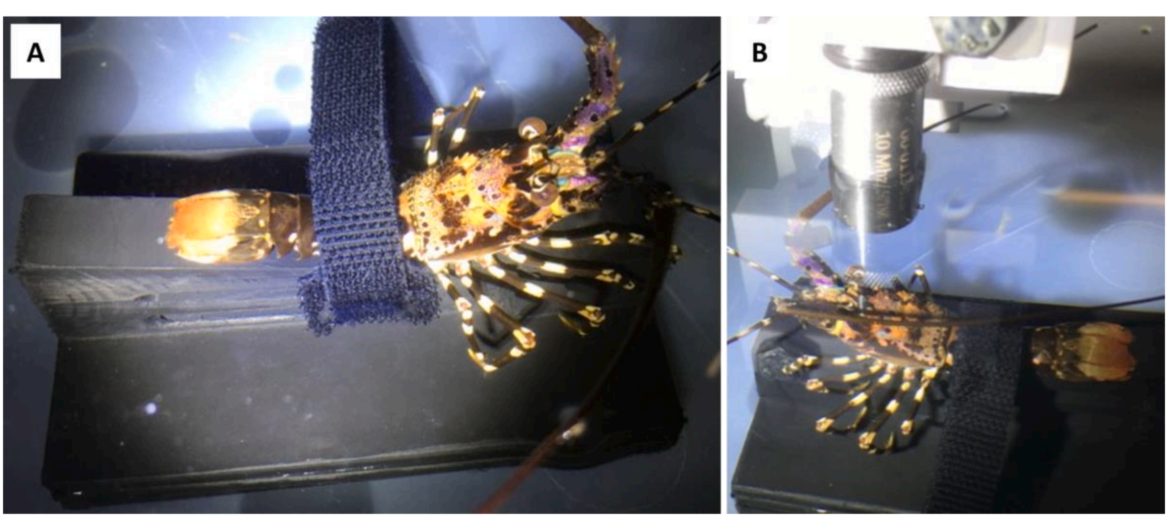

Fig. 2. Juvenile tropical rock lobster *Panulirus ornatus* restrained on an elevated platform for sample acquisition. The water level can be observed on the transducer. A) Restrained lobster; and B) lobster submerged beneath a transducer for sampling.

on the OpBox as this produced an adequate incident pulse and did not risk component failure. Discrete signals were captured with a 100 MHz sampling rate for all transducers.

Gain was set to capture the signal with its highest amplitude approaching the capture limits without saturating. Gain was left unchanged for sampling to ensure comparable values were captured.

3.3. Pulse echo configuration

Using single transducers in pulse echo configuration was the primary focus to acquire scans of lobsters and exuviae for comparison of each transducer. The lobsters were positioned on a weighted high-density polyethylene (HDPE) platform submerged in water for the duration of the scanning process. Exuviae were arranged and positioned on the weighted HDPE platform so that the scan location attained the same position and orientation to the transducer as with lobsters. Each transducer was affixed in turn to an adjustable column to facilitate fine-tuned modifications in height, X and Y-axis translations, and angular adjustments, ensuring optimal alignment perpendicular to the curvature of the lobster's carapace (Fig. 2).

3.4. Through transmission configuration

The S-10 transducer was selected as the transmitting transducer with the signal being focussed on the S-7 receiving transducer as both these transducers were nominally specified for 10 MHz operation. Hard exoskeleton fragments and new integument samples were examined using a through transmission configuration which was required for speed of sound and energy transmission studies. A reference signal of the uninterrupted path was captured. The fragment or sample being tested was placed in the acoustic beam adjoining the receiving transducer and the test signal captured.

4. Preliminary tests

4.1. Lobster exoskeleton thickness

A critical parameter that needs to be established to guide analysis is the thickness of the lobster's exoskeleton. This thickness affects the optimum wavelengths due to the relationship between wavelength and feature resolution along with calculations to determine the speed of sound through the exoskeleton. Fragments of the exoskeleton from intermoult and exuviae groups, and new integument from pre-moult lobsters in the region at the base of the major horns used for acquiring scans were collected and the thickness measured. The fragments were smooth samples of approximately uniform thickness, with underlying connected musculature removed as required. Each fragment's thickness was measured with a micrometer. Care was taken to avoid crushing the samples, especially the new integument due to its softness.

The thickness of hard exoskeleton was measured to be 0.40 ± 0.08 mm with the new integument's thickness measured at 0.40 ± 0.06 mm (Table 2).

4.2. Speed of sound in the exoskeleton of lobsters

As the speed of sound through a medium affects the system's spatial resolution capability, establishing the speed of sound in lobster exoskeleton and the new integument are critical parameters when considering examination through those layers. Determination of the speed of sound through lobster exoskeleton was made and compared between the through transmission and pulse echo configurations, while only through transmission could be used for new integument samples. Acquiring hardened exoskeleton and new integument samples from lobsters required humanely euthanising the lobsters [18].

Table 2
Summary of statistical result data.

Test	n	Mean	SD	Minimum	Maximum	Units
Exoskeleton thickness	9	0.40	0.08	0.3	0.49	mm
New integument thickness	5	0.40	0.06	0.35	0.45	mm
Through transmission speed of sound at 10 MHz (Exoskeleton)	4	3150	590	2250	4180	m/s
Pulse-Echo Transmission of sound (Exoskeleton)	n/a	n/a	n/a	3480	4210	m/s
Through transmission speed of sound at 10 MHz (New integument)	5	1490	190	1310	1850	m/s
Relative through transmission of energy at 10 MHz (Exoskeleton)	6	39.7	8	52.4	30.7	%
Relative through transmission of energy at 10 MHz (New integument)	6	76.0	4.5	81.9	71.7	%
ROI Signal energy (Intermoult)	9	5.9	3.2	1.6	11.1	rel
ROI Signal energy (Pre-moult)	9	2.2	2.1	0.8	7.6	rel
ROI Signal energy (Exuviae)	8	1.8	0.8	0.7	3.0	rel

4.2.1. Establishing a value for the speed of sound in system water

A precision 75 mm micrometre test piece was used to set the distance between the two through transmission transducers so that the speed of sound in the lobster's aquacultural recirculated system could be estimated. The measured speed of sound in the recirculating aquarium water was 1530 ± 10 m/s.

4.2.2. Through transmission speed of sound in lobster exoskeleton and new integument

Placing a sample in the uninterrupted acoustic beam caused a change in delay. Reduced delay resulted in a left shift along the X-axis (delay time) which was considered positive because reduced delay indicates a speed of sound through the sample faster than the coupling water. The sample thickness was also physically measured by micrometre. The speed of sound through the sample in m/s was estimated based on the signal shift caused by sound travelling through the sample thickness with different speed than if traversing the same thickness in water.

$$c_{\mathrm{shell}} = \frac{d}{t_{water} - t_{translocation}} \tag{1}$$

Where t_{water} is the time taken (s) for sound to travel the sample thickness at the speed of sound in water. $t_{translocation}$ is the measured signal delay translocation between the uninterrupted reference signal, and the sample interrupted test signal. d is sample thickness. The speed of sound through hardened lobster exoskeleton is estimated at 3150 ± 590 m/s. The speed of sound through the new integument approximates the speed of sound in sea water and is estimated at 1490 ± 190 m/s (Table 2).

4.2.3. Pulse echo speed of sound in lobster exoskeleton

An irregular recurring feature of high amplitude pulse echo scan envelopes was noted from the S-7, S-10 and O-20 transducers. An initial peak in the envelope was followed by a second lower amplitude peak separated by a deep, steep sided trough. This characteristic was not observed in all scans; however, its noted occurrence saw a common delay of 0.19 to 0.23 μs between the two peaks regardless of the transducer used, indicating independence of signal frequency (Fig. 3). As the second peak is common to the three transducers a common origin for the reflected energy is likely. A reasonable assumption is that the second

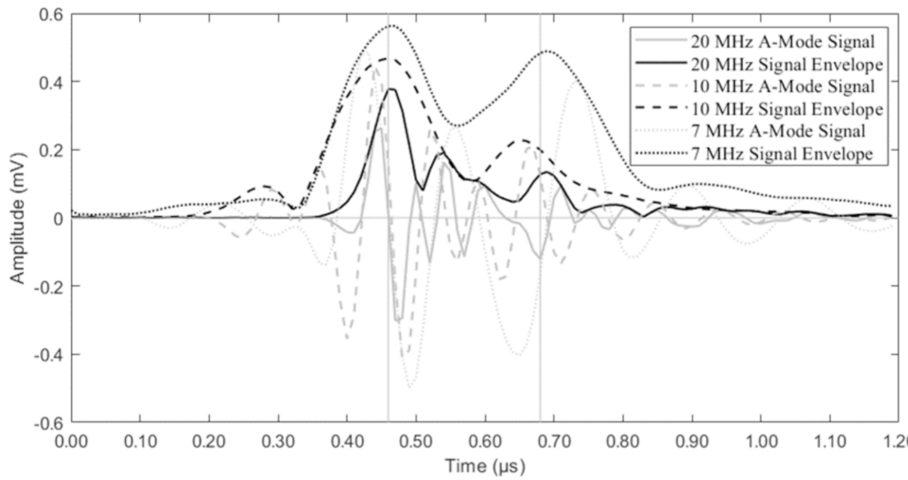

Fig. 3. Scans with signal envelopes taken from different juvenile *Panulirus ornatus* using the S-7 (7 MHz), S-10 (10 MHz), and O-20 (20 MHz) transducers. Each transducer shows an initial peak which are aligned at 0.46 μs and has a vertical line indicating the alignment. The second vertical line is placed with a 0.22 μs delay at 0.68 μs as a reference to illustrate the alignment of a subsequent envelope peak for all three transducers.

peak is a prominent reflection from the inside surface of the exoskeleton due to changing acoustic impedance at this interface. Using the average exoskeleton thickness of 0.40 mm, the measured delay range indicates a speed of sound through the exoskeleton between 3480 and 4210 m/s (Table 2), which is consistent with the range determined in section 4.2.1.

4.3. Ten MHz through transmission of acoustic energy

Understanding the acoustic impediment of the lobster's exoskeleton and new integument are parameters to aid understanding and analysis of the acoustic response. Sufficient residual acoustic energy after exoskeleton penetration will affect the ability to detect layers or structure beneath the exoskeleton. When using a pulse echo configuration, acoustic energy reflected internal of the exoskeleton must be able to penetrate the exoskeleton again to emit the reflected signal to the transducer. In the particular case of pre-moult lobsters, understanding the acoustic impediment of both the exoskeleton and new integument aids understanding the likelihood of the new integument layer generating a characteristic reflection for a solution based on direct echo sounding evaluation of pre-moult.

A through transmission configuration (S-10 transmitting) was used for signal acoustic energy calculations of uninterrupted path and test signals for both hard exoskeleton and new integument samples. Comparative signal energy was calculated using the formula:

$$\sum_{n=i}^{j} v(n)^2 dt \tag{2}$$

v is signal voltage, i and j are the first and last samples of the signal segment, and dt is the 10 ns period between samples (100 MHz sampling rate).

The total signal energy of pulses from the S-10 transducer using constant settings on the pulser/ receiver was captured within an 1800 ns duration envelope defined by each signal's peak amplitude delay minus 600 ns (60 samples) to signal peak amplitude delay plus 1200 ns (120 samples). No signal energy was present outside this duration envelope, hence changing these limits does not change the results. The test signal's energy is expressed as a percentage of the uninterrupted path signal energy. Acoustic energy transmission through hardened lobster exoskeleton was estimated at 39.7 ± 8.0%, with a peak transmission of 52.4% and minimum transmission of 30. 7%. The new integument has acoustic energy transmission of 76.0 ± 4.5% with a maximum transmission of 81.9% and minimum transmission of 71.7% (Table 2).

4.4. Pulse echo testing of the S-7 transducer to establish 7 MHz operation with water coupling

The S-7 transducer was carefully suspended in water to a depth of 2–3 mm so the element was fully submerged, while keeping other critical connections dry. Air bubbles were wiped away from the transducer's surface. A piece of polished stainless steel was used to reflect the signal and the pulser/ receiver set to pulse echo mode. The transducer was aligned to receive its highest amplitude A-mode scan. The OpBox software was then set to display and capture the frequency power curve as a reference for data analysis.

The S-7 transducer was found to have a power output curve with a centre frequency occurring at 7 MHz, shifted from its nominal 10 MHz centre frequency (Fig. 4).

4.5. Transducer operational characteristics

Observations and notes were made on the operational characteristics of each transducer with consideration for the ease with which signals could be attained, and how each transducer performed when sampling the spiny, curved dorsal carapace.

The S-7 transducer produced reliable and repeatable scans, although signal amplitude and form were affected by movement. Signal features remained present and consistent through repeated sampling of each lobster. Signal acquisition was difficult with both the S-10 and O-20 transducers. Signal stability was poor with only small movements resulting in losing the signal entirely. On some occasions it would take minutes of adjusting and moving the lobster through the beam to acquire any signal at all. Considerable perseverance was required to obtain scans using these transducers. When a return signal was acquired, characteristic form, features and delays had a consistent and repeatable appearance. The W-5 transducer produced high scan variability, resulting in an inability to produce consistent, repeatable scans containing identifiable form and features. The S-1 transducer made reproducible scans with a clear reflection from the exoskeleton – water interface. The signal remained intact and consistent with movement of the lobster through the beam.

5. Lobster moult detection

5.1. Transducer representative scans

A key usability criterion for the transducers was generation of consistent scans despite positional changes of the live lobster beneath

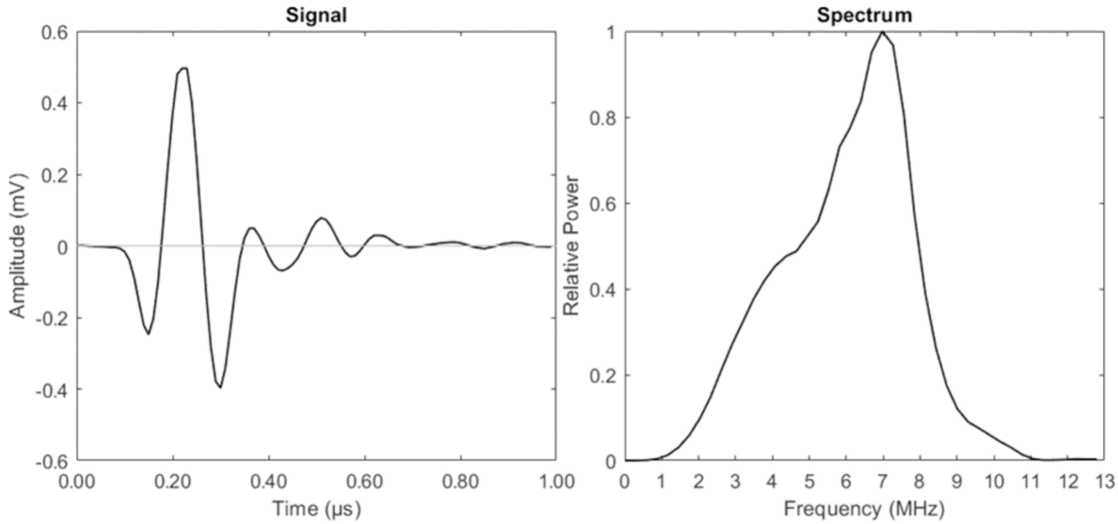

Fig. 4. Captured signal and normalised fast Fourier transform power spectrum as reflected from a polished 316 stainless steel disc coupled through water for the S-7 contact transducer.

the transducer's acoustic beam. Positional changes resulted in scan variations amongst many replicate scans in a sampling session due to the curved and textured dorsal carapace surface. Where a transducer was able to produce mostly similar scans containing consistent signal features, delays, and characteristics despite small positional variations, a single best scan amongst the replicate scans of each lobster in each scanning session was selected to represent that transducer, lobster, and session. The best scan was defined by having characteristics as expected from a good reflection from a smooth surface perpendicular to a damped emitted pulse, i.e., a large amplitude signal with the peak amplitude oscillation occurring with shortest delay. Conversely, where scan variability was high and inconsistent, no single best scan fairly represented the variety of replicate scan results.

Scan replicates from the S-7, S-10, O-20 and S-1 transducers had low variability and each lobster in each session was adequately represented by its best scan (i.e., an intermoult, pre-moult and exuviae representative scan per lobster for each transducer). Scan variability from the W-5 transducer was too high to identify a single best scan to fairly represent the variety of replicate scans and is indicative of a transducer not suited for this operation.

5.2. Pulse echo scan comparisons from the dorsal carapace

The purpose of scan comparisons was to identify signal segments with a consistent, distinguishing difference between the intermoult and pre-moult groups. Exuviae scans acted as a third comparison to accentuate the signal characteristics associated with the ultrasonic acoustic interaction with the exoskeleton. With only water behind the exoskeleton, the exuviae scan helped identify the duration of the exoskeleton reflection only, and allowed a ROI to be determined based on delays succeeding the exoskeleton reflection duration. Penetration beneath the exoskeleton was indicated by acoustic energy having a delay in the intermoult and pre-moult scans clearly exceeding the exoskeleton reflection duration as determined by exuviae scans.

Scan comparisons for distinguishing signal segments were made between the S-7, S-10, O-20 and S-1 transducers because these transducers produced consistent scans. The W-5 transducer was excluded from comparison due to its highly variable scan results. Scans for comparison were aligned by the earliest signal deflection indicating the changing acoustic impedance between the exoskeleton and coupling water. Analytic signals were created using a Hilbert transform of the A-mode and analysed for characteristics of duration, amplitude, and instantaneous phase changes. Scans of each lobster group (i.e., intermoult, pre-moult and exuviae) were overlaid and compared with similarly prepared scans for the three groups.

Analysis of distinguishing signal segments was carried out by defining key delay markers at the start and end of the distinguishing signal segment, thus defining a delay ROI. Signal energy was calculated in the ROI from representative samples from all three lobster groups.

Only the S-7 transducer successfully created a clear distinguishing signal segment. A distinguishing signal segment ROI was identified between 3.30 μs delay and 5.70 μs delay. A low amplitude and long duration signal segment occurring subsequently to the exoskeleton interference duration, which was clear and consistent on exuviae scans, was found in scan samples from intermoult lobsters but was predominantly absent in the same ROI of pre-moult lobsters (Fig. 5). The distinguishing signal segment was also absent from scans of the exuviae.

Signal energy was weakly represented in the ROI of the pre-moult lobsters with 2 pre-moult scans from 8 lobsters having signal amplitude in the ROI. One scan produced a signal segment in the ROI comparable in amplitude and duration to the intermoult scans, while the other scan's signal segment in the ROI was of much reduced duration. There was no signal in the ROI from any exuviae scans.

5.2.1. Statistical check of signal energy in the ROI of the S-7 transducer

The amount of signal energy in the ROI from the S-7 transducer data was calculated using formula 2 from Section 4.3 for each lobster in each of the intermoult, pre-moult and exuviae stage groupings. The data was grouped by stage and the mean and standard deviation of each group calculated and compared.

The ROI mean signal energy check shows a good separation of the intermoult group (5.9 ± 3.2) from both the pre-moult (2.2 ± 2.1) and exuviae groups (1.8 ± 0.8) (Table 2). This encouraging separation of means shows that signal energy was more prevalent in the ROI of intermoult lobsters overall and provides incentive to pursue this research with a more comprehensive experiment and analysis.

5.3. Transducer comparative analysis against a reference S-7 scan

Four transducers were unsuccessful in the task of generating a distinguishing signal segment. To better understand transducer strengths and weaknesses for this application, and so provide information and guidance for further research, comparative analysis of scans from the unsuccessful transducers against the successful S-7 transducer was done. The 7 MHz scan of the intermoult lobster from Fig. 4 was chosen as a comparative reference scan due to having a clear presentation and being

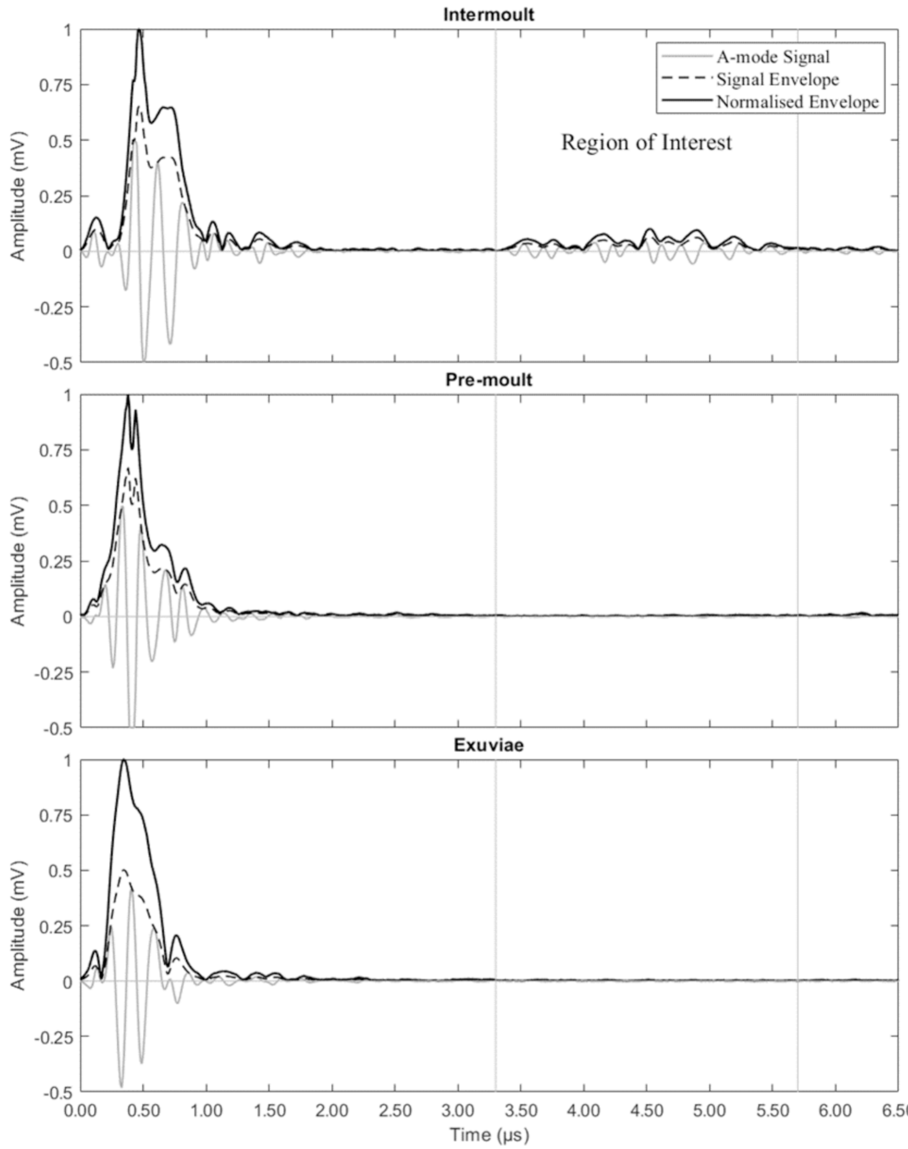

Fig. 5. Comparing representative scans from the S-7 (7 MHz) transducer from the intermoult, pre-moult and exuviae lobster groups. The region of interest defined in the intermoult scan contains a signal segment of long duration and low amplitude that is absent in the same region of the pre-moult and exuviae scans.

representative in delay, duration, amplitude, and waveform in the ROI. Instantiating a reference creates a framework to compare, contrast and interpret signal duration and feature placement of scans from the unsuccessful transducers to better understand their acoustic interaction with the lobsters.

5.3.1. S-10 and O-20 transducers

Comparing the intermoult scans of the S-10 and O-20 transducers with the reference scan showed that both high frequency scans have signal energy being fully attenuated within the exoskeleton signal duration of the reference scan, and prior to the start of the ROI (Fig. 6). The signal energy duration of the intermoult and pre-moult groups was comparable with the exuviae group energy duration. The 10 and 20 MHz scans had no energy beyond the exoskeleton reflection duration as defined by the exuviae scan (Fig. 7). These results corroborate and indicate insufficient acoustic energy penetration beyond the exoskeleton for ultrasonic examination of the tissue adjoining the inside surface of the exoskeleton.

5.3.2. W-5 Transducer

The W-5 transducer was unsuccessful due to high scan variability and an inability to identify representative scans. Nevertheless, an opportunity exists to glean some insights into the functionality of this transducer by comparing select scans against the framework provided by the 7 MHz reference scan.

Two scan variations from each of the three lobster groups were chosen as examples to compare with each other and the 7 MHz reference. Although not definitive, the two scans chosen for intermoult lobsters illustrate that long duration low amplitude signal energy more commonly appears in the ROI defined by the reference scan. This result contrasts with the two pre-moult scans chosen that illustrate short duration energy segments of varying delays that appear both within, and beyond, the ROI. Importantly, energy segments appear in the scans of exuviae after the influence of the exoskeleton has diminished and before, or within, the ROI (Fig. 8). This indicates that this large diameter transducer causes errant reflections of acoustic energy from more distant surfaces of the lobster's curved carapace as the path normal to the transducer through the exuviae only has non-reflecting water behind the exoskeleton.

5.3.3. S-1 transducer

Comparisons of the 1 MHz representative scans with the 7 MHz

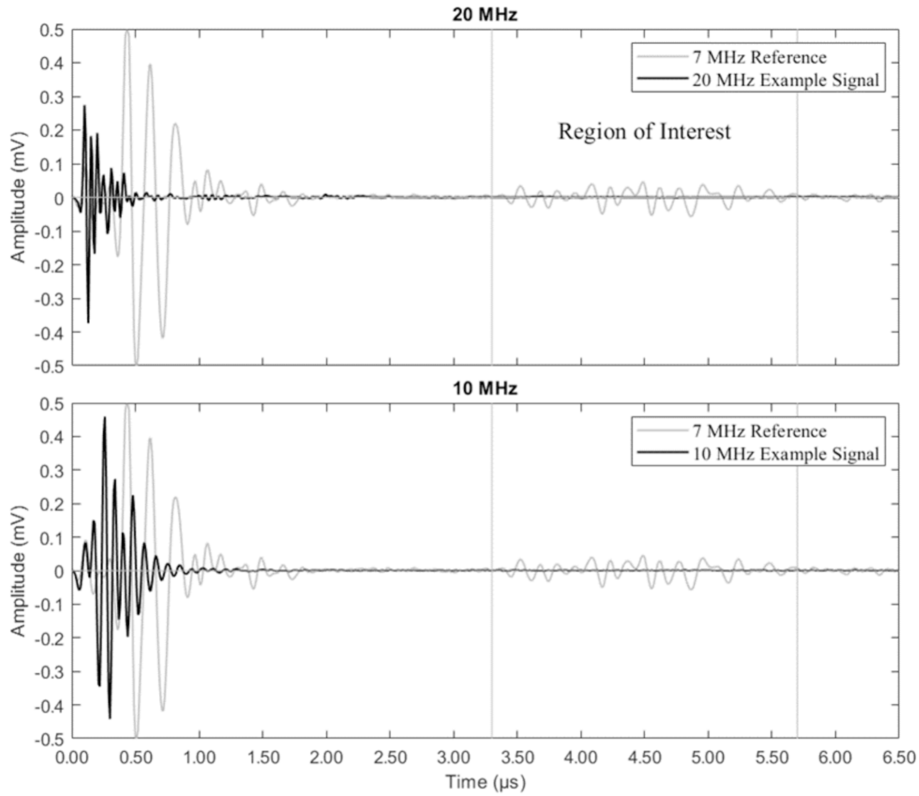

Fig. 6. Comparing scans from the S-10 (10 MHz) and O-20 (20 MHz) transducers with the reference scan from the S-7 (7 MHz) transducer of juvenile *Panulirus ornatus*. The comparison creates a context to examine the signal durations in relation to the ROI defined by the S-7 reference scan. Signal energy is attenuated prior to the ROI for both the S-10 and O-20 transducers.

reference scan show that the 1 MHz centre wavelength of the S-1 transducer is too long to adequately resolve features, with the exoskeleton signal duration exceeding the ROI of the reference signal (Fig. 9). The wavelength of 1 MHz through water with a speed of sound of 1530 m/s (Section 4.2.1) is 1.53 mm which far exceeds the 0.4 mm thickness measured for the lobster's exoskeleton. Deep penetration of acoustic energy with a 1 MHz centre frequency was demonstrated by the observation that test scans taken of the experimental HDPE platform prior to lobster placement showed clear reflections of the platform surface, it's base, and the interface where two HDPE plates were joined. These reflections remained distinct with the lobster placed in the acoustic beam.

6. Discussion

Experimentation with five different NDT transducers used in a pulse echo configuration has yielded intriguing findings and has narrowed future research pathways. Among the transducers tested, the S-7 contact transducer showed the most potential due to the fortuitous alteration of its operational characteristics when used as an immersion transducer, coupling through water. The 7 MHz centre frequency was crucial to gain sufficient signal penetration when compared to the S-10 and O-20 dedicated immersion transducers, while lower frequencies gave insufficient feature resolution due to their longer wavelengths.

The onset of pre-moult is marked by apolysis, the separation of the epidermal cells from the cuticle. Apolysis occurs as the epidermal layer becomes active and starts the process of building the new integument beneath the existing exoskeleton [4,14,15,19,20]. Only the two outermost layers of the new exoskeleton form prior to moulting which leaves the new integument soft so that it can stretch with an active uptake of water into the lobster's body post moult [4,14,15,19–22].

In their pre-moult stage, lobsters experience a physiological transformation, characterized by the growth of a new integument, with the formation of a membrane between the exoskeleton and growing new integument [4,14,15,19,20]. These additional pre-moult layers promoted the consideration of echo sounding as a mechanism of pre-moult detection.

Critical in echo sounding is understanding the likely reflection of acoustic energy from the layers in the sound path. With water as the coupling medium, understanding the acoustic impedance of each layer relative to water gives an indication of the reflectiveness at each layer's interface [23]. The through-transmission studies of speed of sound and energy transmission were required to approximate the acoustic impedance of each surface.

As this study is reporting the speed of sound in any crustacean exoskeleton for the first time, corroborating the speed of sound estimates in the exoskeleton for the through-transmission and pulse-echo methods provided validation for the speed of sound measured. The speed of sound estimated through the exoskeleton based on this observation was determined to be between 3478 and 4210 m s^{-1} based on an average exoskeleton thickness of 0.40 ± 0.08 mm and delays between the peaks between 0.19 and 0.23 μS. These values reasonably correspond with the speed of sound of 3150 ± 590 m s^{-1} estimated in the through transmission study. The estimated range of the speed of sound is large in both cases, which may have possible cause due to high variability in the construction of individual exoskeletons, with the exact composition of each exoskeleton being exposed to environmental, phenotypic, and exogenic influences [24–27]. Sutherland et al. (2023 and 2024) demonstrates high variability observed for carotenoid pigment concentrations in the exoskeleton. High variability of a secreted dorsal surface covering, likely to be calcium carbonate, is also noted [28,29].

Further validation of the speed of sound measurements came from validating the measured speed of sound in the system water, which was found to be 1530 ± 10 m/s. A comparative value from Mackenzie's [30] empirical equation using the measured parameters of the system water

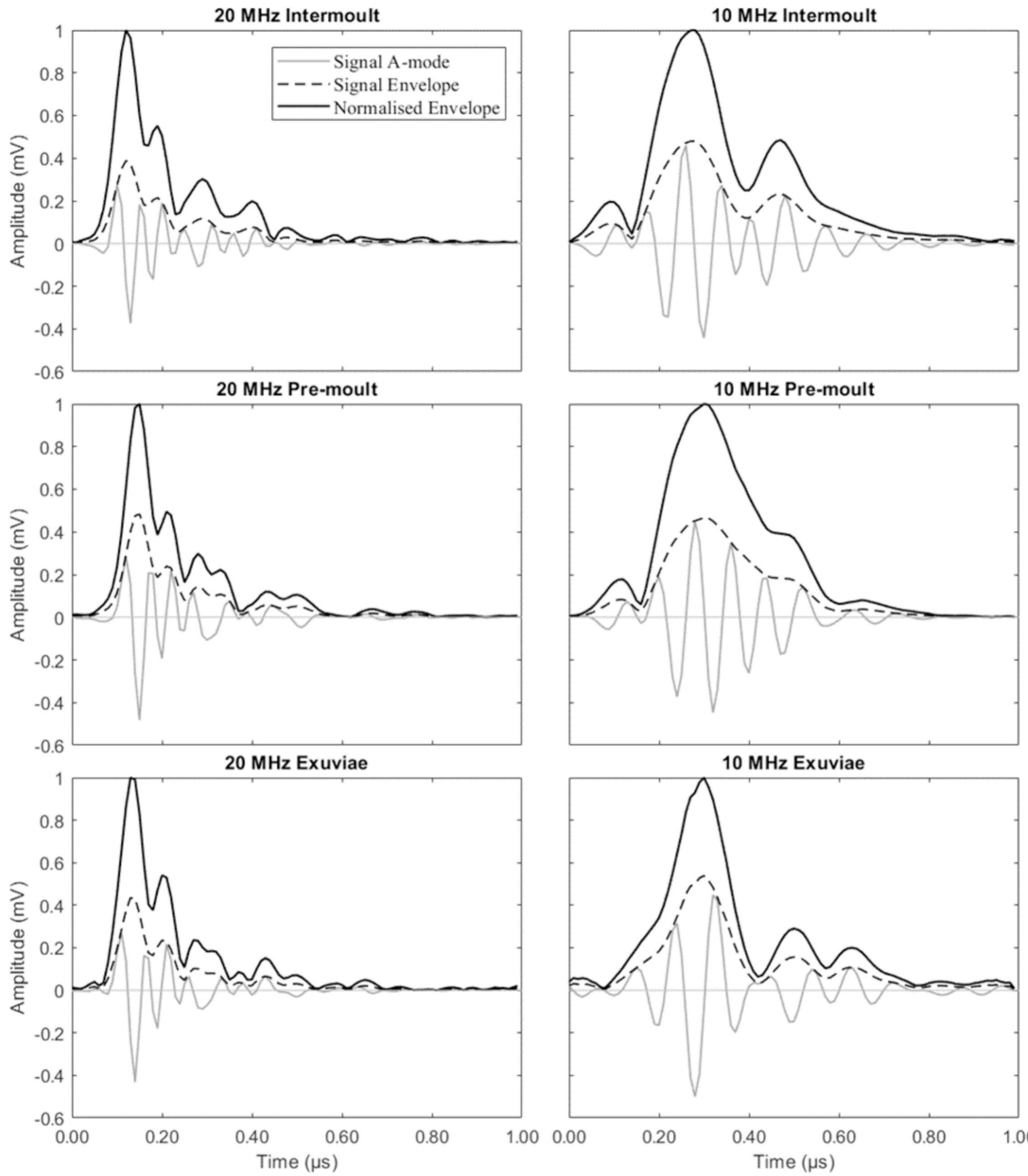

Fig. 7. Representative scans comparing signals and their duration between the intermoult, pre-moult, and exuviae groupings of juvenile *Panulirus ornatus*. The signals and their duration are similarly presented in the exuviae group as they are for intermoult and pre-moult scans for both the O-20 (20 MHz) and S-10 (10 MHz) transducers.

(34 ppt salinity, 26 °C at 0 m depth) for the speed of sound through the system water is 1535.3 m/s.

Confidence of the speed of sound through lobster exoskeleton supports the deduction that the second peak observed in the envelope of scans from the O-20, S-10 and S-7 transducers has its origin from acoustic energy reflected from the inside surface of the exoskeleton. These corroborating results provide confidence that only the through transmission method for measuring the speed of sound in the new integument was sufficient to understand its acoustic impedance for purpose of understanding the likelihood of reflecting sufficient acoustic energy for detection in signal analysis. The through transmission results to establish the speed of sound through the different layers of a pre-moult lobster indicate a large change in acoustic impedance through the hard exoskeleton, while the acoustic impedance of the soft new integument is close to water. Therefore, the reflected energy from the soft new integument is likely to be small, possibly making it invisible for echo sounding [23]. However, a further benefit from the conducting the pulse-echo analysis was insight into the behaviour of acoustic energy within the exoskeleton.

The observation of acoustic reverberation, signal distortion, or signal artefacts after the internal exoskeleton reflection masks the signal segment adjoining the exoskeleton reflection. A similar observation is made by Han et al. [31] when studying the effect of reverberations on near surface NDT imaging through thin films. This masking effect combined with a likely weak reflection from the new integument combines to indicate that echo sounding detection of layers would be unlikely. None of the test transducers could be used for echo sound detection of the additional layers forming beneath the exoskeleton.

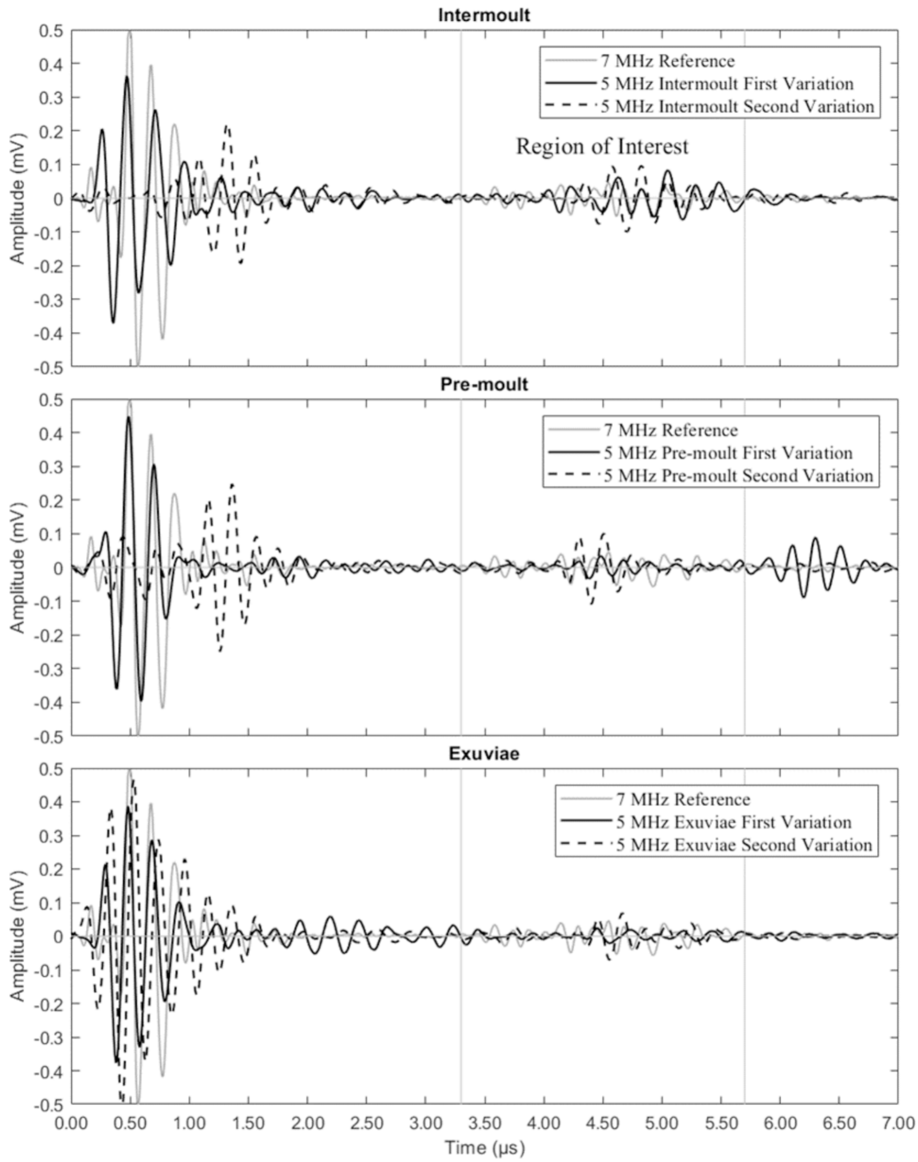

Fig. 8. Comparing scans from the W-5 (5 MHz) transducer with the S-7 (7 MHz) reference scan of juvenile *Panulirus ornatus*. The comparison creates a context to examine variations of signal characteristics, energy distribution and duration in relation to the ROI defined the S-7 reference scan. A signal segment in the ROI for intermoult lobsters from the W-5 transducer is comparable to the S-7 reference scan. Signal energy appears in the pre-moult and exuviae groups, but with haphazardly distributed delays and durations.

A second mechanism for pre-moult detection is indicated by the finding that the S-7 transducer produced a long duration, low-amplitude signal segment by a short delay after the exoskeleton reflection. The delay between the high amplitude exoskeleton reflection and the appearance of the long duration, low amplitude reflection makes this mechanism clearly distinct from the previously discussed echo sounding approach and an internal reflection of acoustic energy from passage through softer tissues is suggest by this observation. This signal segment was predominantly associated with intermoult lobsters and was entirely absent in exuviae scans. The results from testing this transducer provide a compelling indication of a potential mechanism for using ultrasound to detect pre-moult.

Prior to apolysis, the lobster's dormant epidermis is attached directly to the base of the exoskeleton, which firmly anchors internal musculature to the exoskeleton. Post apolysis the underlying musculature becomes separated from the exoskeleton by a membrane and the growing new integument [14,21]. Notably, the changing structure of the exoskeleton during the pre-moult phase appears to influence the ingress and egress of acoustic energy beyond the exoskeleton.

A deeper understanding of the observed distinguishing signal segment can be gleaned from the concept of guided-wave structures. Rose [32] reviews an ultrasonic test for assessing the bond integrity of lap splice joints in older aircraft. Intact joints allowed acoustic energy transmission through the joint, with the acoustic energy traveling to a second receiving transducer. In contrast, debonded joints reflected the acoustic energy back to the source transducer. Drawing parallels to our observations, the appearance of the distinguishing signal segment in intermoult lobsters can be seen as a modified version of the tests that Rose [32] describes. In this context, acoustic energy transmission is facilitated by acoustic coupling provided by musculature directly attached to the base of exoskeleton, creating a mechanism for low amplitude reflections from internal soft tissues to be returned to, and emitted from, the lobster's surface. The acoustic pathway at pre-moult is evidently altered by the membrane and new integument that separate the conductive musculature from the base of the exoskeleton, resulting in acoustic energy either being confined to reverberating within the

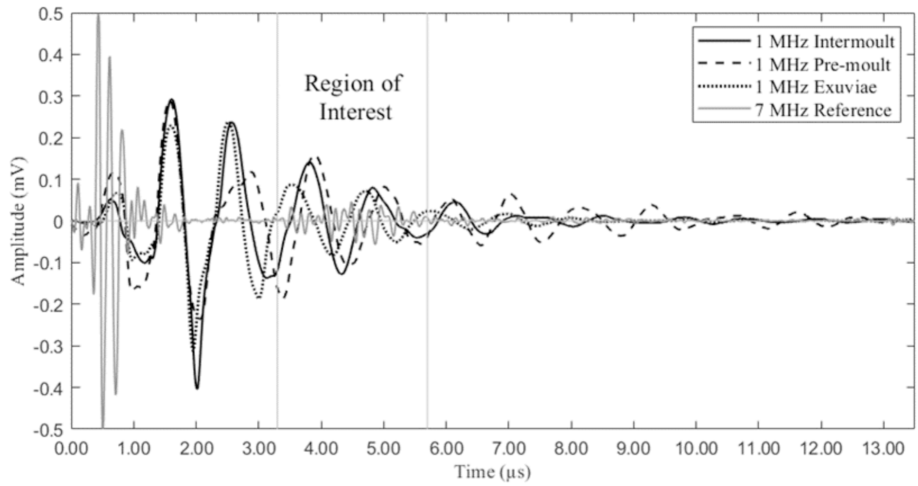

Fig. 9. Comparing representative scans from the S-1 (1 MHz) transducer with the reference scan from the S-7 (7 MHz) transducer of juvenile *Panulirus ornatus*. This comparison creates a context to examine the S-1 transducer signal duration in relation to the ROI defined by the S-7 reference scan. The S-1 wavelength is too long to resolve detail in the ROI.

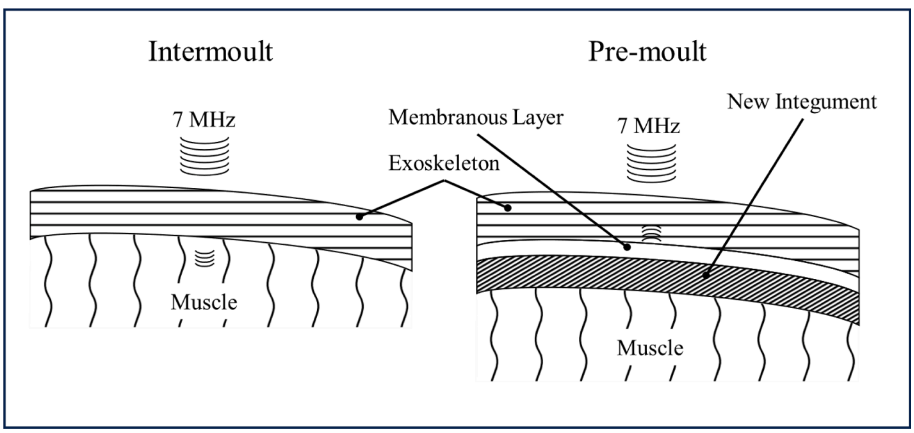

Fig. 10. Illustration of how pre-moult changes beneath the exoskeleton may affect the conduction of acoustic energy. During intermoult, muscle attached to the base of the exoskeleton facilitates acoustic energy transmission. At pre-moult, muscle is separated from the exoskeleton by a membranous layer and the new integument reducing or blocking acoustic energy transmission.

exoskeleton, or internal reflections being unable to return to the lobster's surface (Fig. 10).

Observations of each transducer's ability to produce a stable, usable signal from the lobster's complex textured dorsal carapace indicate an inverse relationship with the transducer's centre frequency. Higher frequencies, such as the S-10 and O-20 transducers, also lacked the penetrative power necessary for this application and this sized lobster. In contrast, the S-1 transducer, while having a wavelength too long for effective resolution, indicated acoustic energy penetration through the entire lobster and into the supporting HDPE platform.

The key observation from the W-5 transducer was that wide area scanning with a large diameter transducer creates high signal variability which limits meaningful interpretation of scan results. Wide area scanning of the complex curvature of the lobster's dorsal carapace and its spiny structure resulted a wide range of possible sound path lengths from numerous reflective surfaces. Using the W-5 transducer to scan exuviae, which have no reflective surfaces beneath the empty exoskeleton, showed sound energy arriving with a delay which would otherwise indicate a reflective source from within the lobster. These results from scans of exuviae were useful in understanding the operation of the W-5 transducer, and showed that overall, no reasonable comparisons between the reference S-7 and W-5 transducers could be made.

Despite these factors indicating uncertainty of the origin of reflected energy, only scans of intermoult lobsters using the W-5 transducer had similar long duration, low amplitude energy signals in the ROI, as was observed using the S-7 transducer. Though uncertainty caused by high variability and haphazardly appearing energy signals extends to all observations in scans made by the W-5 transducer, the possibility exists that the recognisable long duration, low amplitude signals originated inside intermoult lobsters. With the possibility that the 5 MHz centre frequency distinguished intermoult and pre-moult lobsters similarly to the S-7 transducer, further research is suggested using 5 MHz small diameter transducers. Although no firm comparisons or conclusions could be made, the observations from the W-5 transducer remain invaluable as they help refine transducer selection.

The principles of guided-wave structures offer a possible avenue for using ultrasound to differentiate between intermoult and pre-moult lobsters. Further study can now have greater focus and validate this mechanism as a functional discriminator of pre-moult lobsters. The experimental results suggest a centre frequency of 5 to 7 MHz for similar sized juvenile lobsters. The use of higher or lower frequencies for lobsters in other size classes, or for other decapod crustaceans, cannot be excluded. A preference for using unfocused, small diameter elements for scanning small areas is also indicated. A tentative guideline for transducer selection is to choose an unfocused transducer with a diameter approximating the distance between the large anterior horns of the lobster, once they reach a size where this becomes practical. These findings lay a solid foundation for further research into developing an NDT transducer-based pre-moult detector for TRLs and potentially other decapod crustaceans. The potential of ultrasound, when tailored to the unique requirements of this application, could transform the way we approach the challenge of pre-moult detection in lobsters.

7. Conclusion

This study of pre-moult detection using five transducers has delivered valuable insights into acoustic interaction with the exoskeleton of lobsters, and decapod crustaceans more generally. Our observations have feasible explanation using the established acoustic mechanism of guided-wave structure. The aim of this study, to guide future transducer selection, was achieved with a 7 MHz centre frequency generating a signal segment strongly associated with intermoult lobsters. The combination of example transducer specification, distinguishing signal segment, and an established acoustic mechanism greatly reduces experimental scope and focusses further research.

Improved research focus encourages further research using a selection of transducer types around 5 MHz to investigate these preliminary findings and further refine the specifications of the transducer, or transducers. Pulse type and signal processing could also be avenues for future research. Further testing transducers, pulse types, pulser receiver configuration and operation, and signal processing research all contributing towards the creation of an ultrasonic pre-moult detector.

CRediT authorship contribution statement

Charles Sutherland: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Validation, Writing – original draft. **Alan Henderson:** Writing – review & editing, Supervision, Funding acquisition. **Damien Holloway:** Writing – review & editing. **Andrew J. Trotter:** Writing – review & editing, Supervision. **Dean Giosio:** Writing – review & editing, Supervision. **Greg Smith:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

[1] Kelly TR, Giosio DR, Trotter AJ, Smith GG, Fitzgibbon QP. Cannibalism in cultured juvenile lobster *Panulirus ornatus* and contributing biological factors. Aquaculture 2023;576:739883. https://doi.org/10.1016/j.aquaculture.2023.739883.

[2] Kropielnicka-Kruk K, Trotter AJ, Fitzgibbon QP, Smith GG, Carter CG. The effect of conspecific interaction on survival, growth and feeding behaviour of early juvenile tropical spiny lobster *Panulirus ornatus*. Aquaculture 2019;510:234–47. https://doi.org/10.1016/j.aquaculture.2019.05.017.

[3] Marchese G, Fitzgibbon QP, Trotter AJ, Carter CG, Jones CM, Smith GG. The influence of flesh ingredients format and krill meal on growth and feeding behaviour of juvenile tropical spiny lobster *Panulirus ornatus*. Aquaculture 2019;499:128–39. https://doi.org/10.1016/j.aquaculture.2018.09.019.

[4] Aiken DE. Molting and growth. In: The Biology and Management of Lobsters. New York: Academic Press; 1980. p. 91–163.

[5] Dahlgren CP, Staine F. 2007. Growth and survival of Caribbean spiny lobster, *Panulirus argus*, raised from puerulus to adult size in captivity, in: Gulf and Caribbean Fisheries Institute Proceedings. Presented at the Proceedings of the Gulf and Caribbean Fisheries Institute, 59, pp. 303–312.

[6] Vijayakumaran M, Anbarasu M, Kumar TS. Moulting and growth in communal and individual rearing of the spiny lobster. J Mar Biol Assoc India 2010:8.

[7] Kropielnicka-Kruk K, Fitzgibbon QP, Codabaccus BM, Trotter AJ, Giosio DR, Carter CG, et al. The effect of feed frequency on growth, survival and behaviour of juvenile spiny lobster (*Panulirus ornatus*). Animals 2022;12:2241. https://doi.org/10.3390/ani12172241.

[8] Lipcius RN, Herrnkind WF. Molt cycle alterations in behavior, feeding and diel rhythms of a decapod crustacean, the spiny lobster *Panulirus argus*. Mar Biol 1982;68:241–52. https://doi.org/10.1007/BF00409591.

[9] Mills DJ, Verdouw G, Frusher SD. Remote multi-camera system for in situ observations of behaviour and predator/prey interactions of marine benthic macrofauna. N Z J Mar Freshw Res 2005;39:347–52. https://doi.org/10.1080/00288330.2005.9517315.

[10] Sutherland C, Henderson A, Trotter AJ, Giosio D, Smith G. Assessing sensing techniques for detecting markers of approaching ecdysis in juvenile tropical rock lobsters *Panulirus ornatus*. Aqua Eng 2023;102:102342. https://doi.org/10.1016/j.aquaeng.2023.102342.

[11] Haefner PA. Application of ultrasound technology to crustacean physiology; Monitoring cardiac and scaphognathite rates in brachyura. Crustac 1996;69:788–94. https://doi.org/10.1163/156854096X00817.

[12] Gardner C, Rush M, Bevilacqua T. Nonlethal imaging techniques for crab spermathecae. J Crustac Biol 1998;18:64–9. https://doi.org/10.1163/193724098X00070.

[13] Macrì F, Di Pietro S, Bonfiglio R, De Stefano C, Giorgianni P, Bottari T. Anatomical evaluation of the organs in the red swamp crayfish, *Procambarus clarkii*, by diagnostic ultrasound examination. J Crustac Biol 2013;33:586–9. https://doi.org/10.1163/1937240X-00002156.

[14] Roer R, Dillaman R. The structure and calcification of the crustacean cuticle. Am Zool 1984;24:893–909. https://doi.org/10.1093/icb/24.4.893.

[15] Travis DF. The molting cycle of the spiny lobster, *Panulirus argus* Latreille. II. Pre-ecdysial histological and histochemical changes in the hepatopancreas and integumental tissues. Biol Bull 1955;108:88–112. https://doi.org/10.2307/1538400.

[16] Drach P. Mue et cycle d'intermue chez les Crustacés Décapodes. Ann Inst Oceanogr Paris 1939:103–391.

[17] Turnbull CT. Pleopod cuticular morphology as an index of moult stage in the ornate rock lobster, *Panulirus ornatus* (Fabricius 1789). Mar Freshw Res 1989;40:285–93. https://doi.org/10.1071/mf9890285.

[18] Atherley NAM, Freeman MA, Dennis MM. Post-mortem examination of the Caribbean spiny lobster (*Panulirus argus*, Latreille 1804) and pathology in a fishery of the Lesser Antilles. J Invertebr Pathol 2020;175:107453. https://doi.org/10.1016/j.jip.2020.107453.

[19] Hadley NF. The arthropod cuticle. Sci Am 1986;255:104–13.

[20] Passano LM. Molting and its control. In: Waterman TH, editor. The Physiology of Crustacea. New York: Academic Press; 1960. p. 473–536. https://doi.org/10.1016/B978-0-12-395628-6.50021-X.

[21] Travis DF. The molting cycle of the spiny lobster, *Panulirus argus* Latreille. I. Molting and growth in laboratory-maintained individuals. Biol Bull 1954;107:433–50. https://doi.org/10.2307/1538591.

[22] Travis DF. The molting cycle of the spiny lobster, *Panulirus argus* Latreille. IV. Post-ecdysial histological and histochemical changes in the hepatopancreas and integumental tissues. Biol Bull 1957;113:451–79. https://doi.org/10.2307/1539076.

[23] Kuttruff H. Acoustics: An introduction. Taylor & Francis; 2007.

[24] Aiken D, Waddy S. The growth process in crayfish. Rev Aquat Sci 1992;6(3,4):335–81.

[25] Greenaway P. CALCIUM BALANCE AND MOULTING IN THE CRUSTACEA. Biol Rev 1985;60:425–54. https://doi.org/10.1111/j.1469-185X.1985.tb00424.x.

[26] Lowder KB, deVries MS, Hattingh R, Day JMD, Andersson AJ, Zerofski PJ, et al. Exoskeletal predator defenses of juvenile California spiny lobsters (Panulirus interruptus) are affected by fluctuating ocean acidification-like conditions. Front Mar Sci 2022;9.

[27] Tlusty M, Hyland C. Astaxanthin deposition in the cuticle of juvenile American lobster (Homarus americanus): implications for phenotypic and genotypic

coloration. Mar Biol 2005;147:113–9. https://doi.org/10.1007/s00227-005-1558-0.

[28] Sutherland C, Henderson A, Trotter AJ, Giosio D, Smith G. 2024. Sensing pre and post ecdysis of tropical rock lobsters, *Panulirus ornatus*, using low-cost components and novel spectral camera. Manuscript under review for publication in J. Mar. Sci. Eng.

[29] Travis DF, Friberg U. The deposition of skeletal structures in the crustacea: VI. Microradiographic studies of the exoskeleton of the crayfish Orconectes virilis hagen. J Ultrastruct Res 1963;9:285–301. https://doi.org/10.1016/S0022-5320(63)80008-8.

[30] Mackenzie KV. Nine-term equation for the sound speed in the oceans. J Acoust Soc Am 1981;70(3):807–12. https://doi.org/10.1121/1.386920.

[31] Han Y, Yang K, Chen J, Wu E, Jin H. Ultrasonic imaging through reverberation media. Ultrasonics 2023;131:106959. https://doi.org/10.1016/j.ultras.2023.106959.

[32] Rose JL. 1995. Recent advances in guided wave NDE, in: 1995 IEEE Ultrasonics Symposium. Proceedings. An International Symposium. Presented at the 1995 IEEE Ultrasonics Symposium. Proceedings. An International Symposium, pp. 761–770 vol.1. https://doi.org/10.1109/ULTSYM.1995.495679.