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RESEARCH ARTICLE

K. J. Wright · D. M. Higgs · A. J. Belanger · J. M. Leis

Auditory and olfactory abilities of pre-settlement larvae and post-settlement juveniles of a coral reef damselfish (Pisces: Pomacentridae)

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Abstract The propagules of most species of reef fish are advected from the reef, necessitating a return to reef habitats at the end of the pelagic stage. There is increasing evidence of active attraction to the reef but the sensory abilities of reef fish larvae have not been characterized well enough to fully identify cues. The electrophysiological methods of auditory brainstem response (ABR) and electroolfactogram (EOG) were used to investigate auditory and olfactory abilities of pre- and post-settlement stages of a damselfish, *Pomacentrus nagasakiensis* (Pisces, Pomacentridae). Audiograms of the two ontogenetic stages were similar. Pre-settlement larvae heard as well as their post-settlement counterparts at all but two of the tested frequencies between 100 Hz and 2,000 Hz. At 100 and 600 Hz, pre-settlement larvae had ABR thresholds 8 dB higher than those of post-settlement juveniles. Both stages were able to detect locally recorded reef sounds. Similarly, no difference in olfactory ability was found between the two ontogenetic stages. Both stages showed olfactory responses to conspecifics as well as L-alanine. Therefore, the auditory and olfactory senses have similar capabilities in both ontogenetic stages. Settlement stage larvae of *P. nagasakiensis* can hear and smell reef cues but it is unclear as to what extent larvae use these sounds or smells, or both, as cues for locating settlement sites.

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K. J. Wright (✉)
School of Biological, Earth and Environmental Sciences,
University of New South Wales, Sydney, NSW 2052, Australia
E-mail: kjwright_@hotmail.com

D. M. Higgs · A. J. Belanger
Department of Biology, University of Windsor,
Windsor, ON N9B3P4, Canada

J. M. Leis · K. J. Wright
Ichthyology and Division of Aquatic Zoology,
Australian Museum, 6 College St, Sydney,
NSW 2010, Australia

Introduction

Coral reef fish have a bipartite lifecycle, wherein the first few days to months of life are spent as a larva in the pelagic environment, feeding, developing and growing before it is necessary to locate a suitable reef habitat for settlement (Leis 1991; Leis and McCormick 2002). Once a suitable benthic settlement habitat is located, a larva must metamorphose, thus changing both habitat and morphology over a short time period. Knowledge of the key processes and behaviours over this transition period are vital for understanding the life history of reef fishes and for the management of populations. Many gaps that had previously existed in our knowledge of behaviour and processes over this time are only recently being filled and this paper continues by investigating two sensory abilities of a pomacentrid damselfish over the settlement transition.

The dispersal of reef fish larvae was long believed to be passive, with settlement thought to occur wherever currents took the larvae (Roberts 1997). These assumptions of passive dispersal are being revised, and strong evidence now exists to dispel the hypothesis of coral reef fish larvae as being only passive. Settlement-stage larvae have strong swimming abilities, are able to swim at speeds greater than ambient currents, can swim long distances and can change both their horizontal and vertical trajectories (Leis et al. 1996; Leis and Carson-Ewart 1997, 2003; Stobutzki and Bellwood 1997). Larvae are also able to orientate in the pelagic environment (Stobutzki and Bellwood 1998; Leis and Carson-Ewart 1999, 2003) and return of larvae to natal reefs is now known to occur, in some cases accounting for as much as 60% of all new recruits (Jones et al. 1999; Swearer et al. 1999; Taylor and Hellberg 2003). Thus, larvae are far from passive and sensory abilities may play a vital role in their dispersal and settlement location.

The sensory abilities of coral reef fish larvae were previously assumed to be poor, being developed suffi-

ciently for feeding and little else (Myrberg Jr and Fuiman 2002). We now know that this is not the case. Light traps deployed at night with a speaker broadcasting nocturnal reef noise attract more larvae than light traps that are quiet (Tolimieri et al. 2000; Leis et al. 2003) and the behaviour of larvae is altered in the presence of broadcast nocturnal reef noise (Leis et al. 2002). Further, larvae are able to distinguish between artificial sounds (pure tones) and natural sounds (reef noise) (Leis et al. 2002) and have the ability to localize a sound source (Tolimieri et al. 2004; Leis and Lockett 2005). In addition, the hearing sensitivity of post-settlement juveniles of two damselfishes showed converse changes with size—auditory thresholds of *Stegastes partitus* decreased with increasing size (Kenyon 1996) whilst thresholds of *Abudefduf saxatilis* increased with increasing size (Egner and Mann 2005), although no data exist on either species responses prior to settlement. The sense of smell has also been shown to be functional and utilised by settlement-stage reef-fish larvae. Apogonid larvae are capable of distinguishing between lagoon water and oceanic water by olfaction (Atema et al. 2002), and larval anemonefish use olfaction to locate a host anemone (Elliot et al. 1995; Arvedlund et al. 1999). All of these studies, however, are behavioural and not physiological and none have examined sensory abilities during the pelagic to benthic transition.

The present study aimed to determine electrophysiologically, the auditory and olfactory abilities of a coral reef damselfish, *Pomacentrus nagasakiensis*, at settlement stage to identify potentially relevant cues for reef attraction. We also examine if the sensitivity of these senses changed with settlement and concomitant metamorphosis. Pomacentrids are a dominant feature of coral reef communities—they are one of the most speciose of fish families on coral reefs and account for a large proportion of the individual and fish biomass on coral reefs (Leis and Carson-Ewart 2002). Like most species of damselfishes (Allen 1991), *P. nagasakiensis* has a bipartite life cycle, with a pelagic larval stage followed by settlement on suitable reef habitat for the adult benthic phase of the life cycle (McCormick et al. 2002). To test auditory and olfactory abilities across the settlement transition, we employed two physiological methods previously used in auditory and olfactory tests in fish—auditory brainstem response (ABR) (Kenyon et al. 1998; Yan and Curtsinger 2000; Higgs et al. 2003; Wysocki and Ladich 2003) and electroolfactogram (EOG) (Caprio 1978). Both techniques measure electrical responses evoked by the sensory stimuli of sound and smell, and are a direct evaluation of auditory and olfactory ability, respectively. This is the first study to use ABR to test the hearing sensitivity of coral reef fish larvae, with previous work being confined to freshwater species or benthic juveniles and adults. It is also the first to use the EOG technique for the larvae of a marine fish. Using physiological techniques allow us to ascertain whether natural reef stimuli are a viable cue for orientation to settlement habitats and begin to quantify minimal stimulus levels required to drive such responses.

Materials and methods

Pre-settlement stage larvae were caught using a light trap in open water off Lizard Island on the Great Barrier Reef (14°40′S, 145°27′E), and examination of sensory abilities took place on the day of capture. Some of the *P. nagasakiensis* larvae were kept in aquaria that contained coral rubble as a substrate and were fed newly hatched *Artemia salina* naupli for at least 14 days before testing, so they could undergo metamorphosis. This was done to assure a steady supply of post-settlement fishes for testing. During this time, the typical suite of morphological changes that accompany metamorphosis took place—colour change from grey to blue and changes to snout angle, dorsal and pectoral spine length and body depth (McCormick et al. 2002). All fish tested were wild-caught and had been exposed to the auditory and olfactory stimuli in the field for approx. 3 weeks prior to capture in the light trap.

Auditory physiology

Auditory abilities of pre- and post-settlement *P. nagasakiensis* were examined using ABR. Originally used in mammalian audition studies, the technique has been adapted for audition studies on fishes (Corwin et al. 1982; Kenyon et al. 1998). ABR is ideal for the study of larval fish audition as it is can provide an auditory assessment for fragile species. No behavioural conditioning or training of an animal is required for ABR, and it allows rapid measurement of hearing capability.

We tested 12 pre-settlement and 9 post-settlement fish. The pre-settlement larvae ranged in size from 12 mm to 15 mm standard length (SL) and the post-settlement juveniles were from 15 mm to 17 mm SL. We adapted the methods of Higgs et al. (2003) for all ABR work. Larvae were positioned on their side on clay resting on a Perspex slide attached (at a perpendicular angle) to a plastic pipette. Larvae were held in place by metal staples positioned around their bodies. A micromanipulator was used for fine positioning of the fish holder. Measurements were taken underwater in a tank constructed from PVC (5 mm thick) pipe 0.25 m in diameter and 1.17 m long (lying horizontally) with an opening of 1 m by 0.15 m, allowing a free water surface area of roughly 0.15 m^2. An underwater speaker (University Sound UW-30) was placed vertically at one end of the tank, 0.76 m from the fish holder located at approximately half the tank depth (0.12 m). Fish were completely submerged with the head approximately 10 cm below the water surface. The water temperature of the tank was maintained at the same temperature as the holding tank in which the fish were kept (24°C). No air bubblers were used in the holding tank to avoid

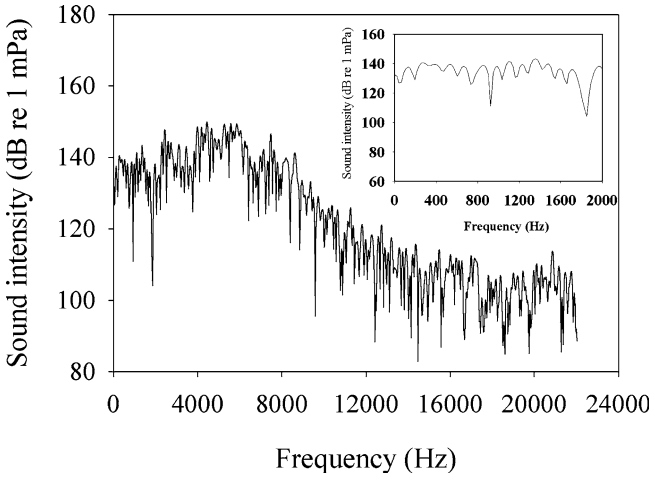

Fig. 1 Fast Fourier Transformation (FFT) of 10 ms reef sound recorded over Vicki's Reef near Lizard Island on the Great Barrier Reef at night. FFT size was 2,048 with a sample rate of 48,000 Hz

damage to the fish's hearing (a flow-through seawater system kept water aerated). No anaesthetics or muscle relaxants were used in these experiments. As a control, dead fish were also tested in the apparatus, and at no time did a dead fish produce a response similar to the responses of the experimental animals (see Fig. 2).

Fig. 2 Auditory brainstem response traces to an (**a**) 100 Hz tone burst, (**b**) 600 Hz tone burst and (**c**) segment of raw coral reef noise recorded over the reef at night. The *bars* under the waveforms indicate stimulus timing. The *arrow* indicates the position of the response. All intensities are expressed as dB re 1 µPa. Auditory threshold, or the lowest SPL to show a definite response, occurred at 110 dB for 100 Hz, 125 dB for 600 Hz and at 157 dB for night reef noise in these examples. No response was seen for the dead controls (bottom trace in **a**, **b** and **c**)

Auditory stimuli were presented using a Tucker-Davis Technologies (TDT, Gainesville, FL, USA) physiological apparatus controlled by a computer running TDT SigGen (Version 4.4) and BioSig (Version 4.4) software. The underwater speaker was attached to the TDT apparatus and tone bursts (10 ms in duration with a 2 ms rise–fall time gated through a Hanning Window) with frequencies of 100, 200, 300, 400, 500, 600, 700, 800, 1,200 and 2,000 Hz were played, covering the expected range of fish hearing (Myrberg Jr and Spires 1980; Fay and Megala Simmons 1999). Acoustic intensities were calibrated using a HighTech Inc HTI-96 Min Series hydrophone (sensitivity −163.7 dB V/1 uPa) placed in the fish holder before experiments were begun. Sound levels at each frequency were measured on a digital oscilloscope (Tektronix TDS 1002) and adjusted so that BioSig would output the desired decibel levels. Segments of night reef noise (random section of a recording from Vicki's Reef at Lizard Island) were also played in 10 ms bursts. The Fast Fourier Transformation (FFT) of the night sound stimuli showed a largely flat response until around 8,000 Hz, with most of the energy concentrated below 6,000 Hz (Fig. 1). At each frequency, sound intensity was increased in 5 dB increments until a stereotypical ABR was observed. Measurements were continued to at least 10 dB above threshold to examine suprathreshold levels. Owing to speaker limitations, not all frequencies could be played to the same sound pressure level (SPL). Therefore, the maximum SPL was 150 dB re 1 µPa from 100 Hz through to 500 Hz, and 2 kHz, 145 dB re 1 µPa for 600 and 800 Hz, and 140 dB re 1 µPa for 700 Hz and 1.2 kHz (Table 1).

Auditory brainstem responses were collected using two stainless steel sub-dermal electrodes (Rochester Electromedical Inc., Tampa, FL, USA). Each electrode

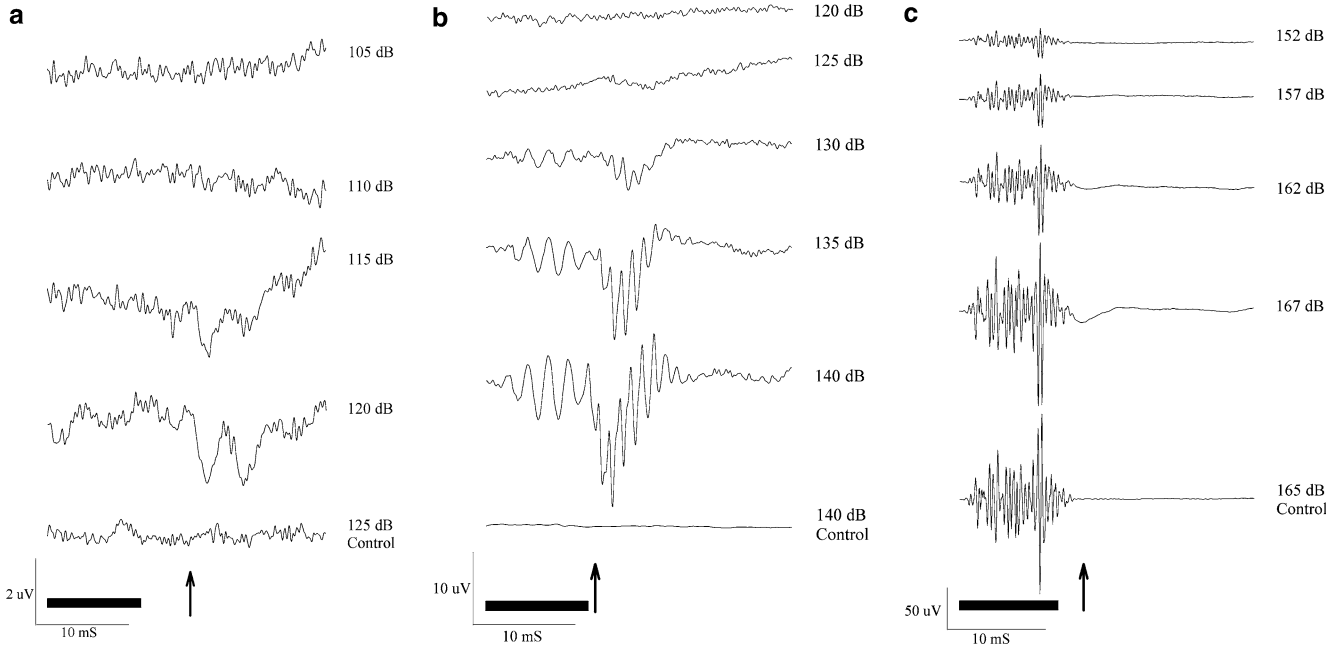

Table 1 Summary of all ABR data for pre- and post-settlement individuals. The maximum SPL level is given for each frequency (see Methods for further details)

Frequency (Hz)	dB level	Pre-settlement		Post-settlement	
		Response	No response	Response	No response
100	150	8	0	7	0
200	150	8	0	5	0
300	150	7	0	3	0
400	150	7	0	4	0
500	150	7	0	4	0
600	145	9	0	8	0
700	140	7	0	3	0
800	145	5	2	5	1
1,200	140	1	4	1	2
2,000	150	2	5	2	5
Night reef	170	7	0	4	0

was covered in nail varnish for insulation, except the tip, and was positioned by a micromanipulator. The electrodes were placed subcutaneously, with the tip of the electrode just penetrating the skin. The recording electrode was positioned dorsally, just posterior to the operculum. The reference electrode was positioned dorsally in the nasal region. Responses were obtained from 200 stimulus presentations at each intensity and frequency (100 from stimuli presented at 90° phase and 100 from stimuli presented at 270° phase) and averaged to cancel stimulus artefacts.

Auditory threshold was defined as the lowest level at which a clear response could be detected. Detection of the auditory threshold was done visually, which has been shown to produce identical results to the use of statistical approaches (Mann et al. 2001). Examples of stereotypical ABR can be seen in Fig. 2.

Olfactory physiology

Olfactory ability of pre- and post-settlement *P. nagasakiensis* individuals was determined using EOG. The EOG technique measures olfactory transduction by recording the change in the negative electrical potential at the surface of the nasal epithelium. Like ABR, EOG is a non-invasive technique suitable for measuring the olfactory abilities of a fragile larval fish.

Six pre-settlement (12–15 mm SL) and seven post-settlement larvae (13–15 mm SL) were tested using EOG procedures similar to those used in other studies (Caprio 1978; Moore and Waring 1996; Murphy et al. 2001). Briefly, the larvae were wrapped gently in a wet piece of Kimwipe and restrained on their side for the procedure on a Perspex stand. No anaesthetics or muscle relaxants were needed. A tube placed in front of the mouth continually delivered an oxygenated water flow through the mouth and over the gills (this water did not flow over the nose). All EOG experiments were conducted in a Faraday cage constructed of steel mesh in order to block out background electrical noise by grounding the cage.

Electroolfactogram responses were recorded by two stainless steel electrodes (Rochester Electromedical Inc., Tampa, Florida). All exposed surfaces of the electrodes were insulated with nail varnish. Electrodes were positioned using micromanipulators, with the recording electrode being inserted into the excurrent hole of the nostril and the reference electrode placed approximately 1 cm away from the nostril on the ipsilateral side of the skin. An odour delivery tube positioned over the posterior nostril constantly perfused the nasal cavity with 24°C seawater from the Lizard Island Research Station seawater system (background flow). The test solution was manually switched from the background for a period of 5 s when testing occurred. No change in flow rate occurred during this switch, eliminating the possibility of a mechanical response. An oscilloscope (Tektronix TDS1002) and computer with Wavestar software (Version 2.6) were used to document and record the amplified response (Grass-Telefactor CP122 Amplifier). Each individual was only tested once.

The test stimulant for this study was conspecific conditioned seawater (CW), obtained as follows. Fifteen randomly selected juveniles were placed in 300 ml of seawater from the Lizard Island Research Station seawater system with an airstone for a period of 1 h. The CW was used undiluted within 3 h of preparation. The amino acid L-alanine was used to test functioning of the EOG preparation and allowed us to monitor the stability of the recording throughout the experiment, as all fish have been found to be sensitive to amino acid standards (Hara 1992). All test odours were prepared using the background water and maintained at the same temperature, ensuring that any response noted was not due to water or temperature differences. Olfactory acclimation was minimised by allowing at least 2 min between exposures to test solutions. In addition, dead fish controls were run, as well as controls where the recording electrode was placed on a different part of the body. At no time did control traces resemble positive responses (see Fig. 5). Data (base to peak voltage differences) are presented as an absolute mV response.

Statistical analyses

As the number of test subjects were dependent upon light-trap catches, the datasets for pre- and post-settlement fish were unbalanced. Therefore, Generalised Linear Modelling was used to compare auditory and

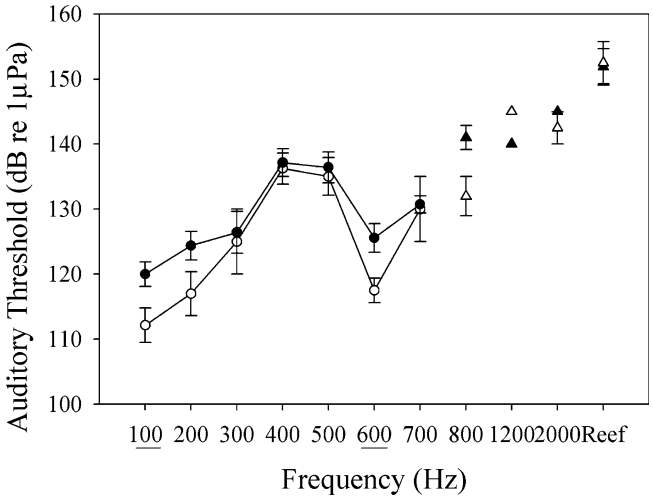

Fig. 3 Auditory thresholds for pre- (*solid symbols*) and post-settlement (*open symbols*) fish. Values are means ± standard errors. Responses for frequencies greater than 700 Hz are represented by *triangles*, indicating that these measurements are minimum estimates due to equipment limitations (see Methods). *Underlined frequencies* indicate a significant difference ($P < 0.05$) between pre- and post-settlement responses

olfactory sensitivity (separately) between the two groups. Where significant differences were found, Bonferroni post hoc tests were conducted. For all tests, the significance level was $\alpha = 0.05$.

Results

Auditory physiology

The ABR waveform shape produced in response to sound was dependent on the frequency. Waveforms produced in response to 100 and 200 Hz tone bursts displayed two waves in the first 20 ms (Fig. 2a). For frequencies of 300 Hz and above, the waveforms appeared as one wave, dropping away from the horizontal in response to the stimulus before rising quickly again (Fig. 2b). The response to the segment of coral reef noise was a shallow dip from the horizontal at the offset of the stimulus (Fig. 2c).

Audiograms for pre- and post-settlement fish were similar (Fig. 3) with the most sensitive frequencies consistent across the two stages. For both pre- and post-settlement fish, threshold was lowest (sensitivity highest) at 100 Hz, followed by 200 and 600 Hz (Fig. 3). The auditory threshold for both pre- and post- settlement fish increased from 100 Hz to 400 and 500 Hz, and then dropped at 600 Hz. Thereafter, thresholds increased with increasing frequency from 700 Hz to 2,000 Hz. Both pre- and post- settlement fish were least sensitive to night reef noise.

The hearing of post-settlement fish was significantly more sensitive than their pre-settlement counterparts at only two frequencies: 100 Hz ($P = 0.028$) and 600 Hz ($P = 0.010$) (Fig. 3). Thresholds of post-settlement fish were 8 dB lower than thresholds of pre-settlement fish at these two frequencies. Frequencies above 700 Hz provided minimal threshold estimates (due to some fish having "no response" to the maximum SPL but would have presumably shown a response if the SPLs could have been increased) and were excluded from formal statistical analysis due to the speaker limitations described in the methods, but for higher frequencies only at 800 Hz was there any indication of a possible difference in sensitivity between pre-and post-settlement fish (Fig. 3).

Significant differences in sensitivity were found among frequencies (Fig. 4). The auditory threshold for the raw segment of night coral reef noise was significantly higher than all other frequencies for both pre- and post-settlement fish. The relative ranking of sensitivities among frequencies was the same for both pre- and post-settlement individuals. Frequency groupings of pre-settlement fish were largely overlapping, whereas the frequency groupings for post-settlement fish were more discrete with sensitivity to reef noise significantly less at 300, 400, 500 and 700 Hz and sensitivity to 100, 200 and 600 Hz were significantly greater.

Olfactory physiology

Olfactory response waveforms were typical of EOG waveforms (Fig. 5), rising sharply and then declining

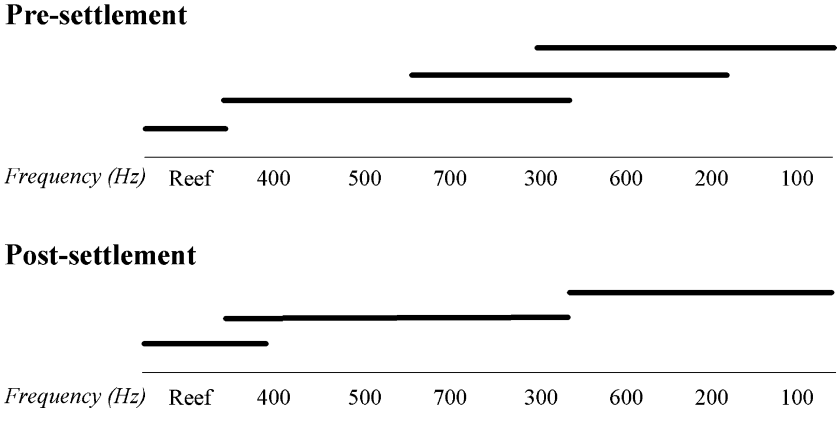

Fig. 4 Frequency groupings according to similarity of detection by pre- and post-settlement fish. Frequencies are arranged in order of decreasing fish hearing sensitivity. Frequencies linked by a *horizontal line* do not differ significantly in sensitivity from other frequencies along the same line. *Separate lines* indicate significant differences

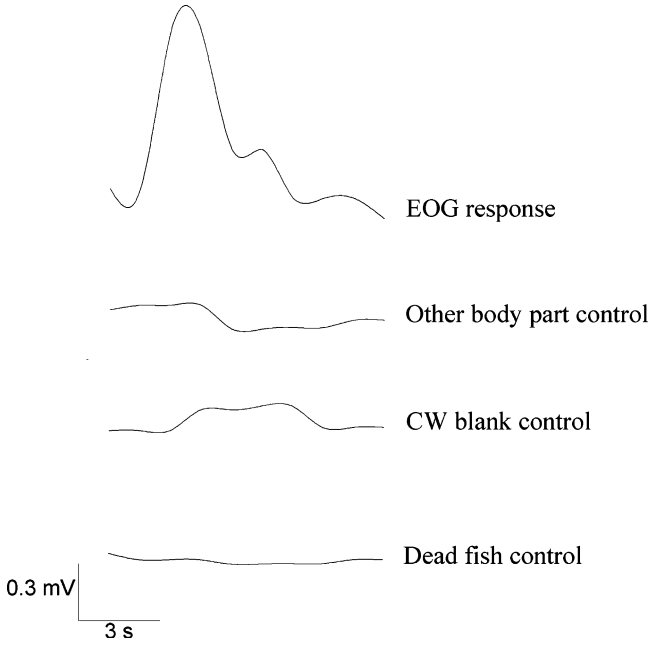

Fig. 5 Typical EOG waveforms (mV) produced in response to odour (L-alanine) (responses to CW were the same)

gradually as the odour washed away. All of the responses recorded for both pre- and post-settlement fish to both L-alanine and CW were significantly greater than the background level (Fig. 6). The olfactory responses of pre- and post-settlement fish were the same in response to the amino acid L-alanine. The EOG response to CW was larger for pre-settlement fish than post-settlement fish, but the difference was not significant ($P = 0.07$).

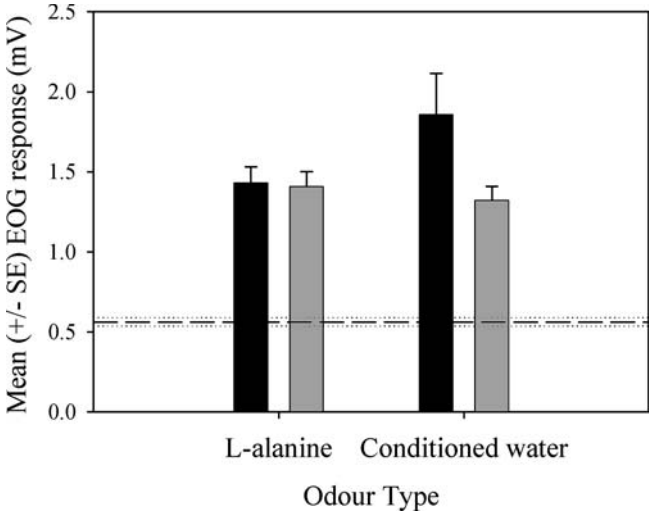

Fig. 6 Mean (±SE) EOG responses (mV) to odour stimulants for pre- (*black bars*) and post-settlement (*grey bars*) fish. *Dashed line* indicates the average background level (mV) and *dotted lines* indicate the standard error

Discussion and conclusions

Auditory physiology

Although audiograms are presented here in terms of sound pressure, in reality fish react to both the pressure and displacement components of sound waves, with the contribution of each component varying with frequency, distance to source, and any specialisations present within the species of interest (Fay and Megala Simmons 1999). It would have been preferable to estimate thresholds for both sound pressure and particle displacement, but this was not possible in our current study. It is theoretically possible to estimate the particle displacement from the acoustic pressure but this assumes an ideal water body with no boundaries or interfering particles (Rogers and Cox 1988). Thus, the audiogram information presented here, or published anywhere, is not an absolute minimum threshold for hearing but rather represents a first approximation that can be compared to the audiograms of other species using similar techniques (Higgs 2002). The sensitivities are therefore a conservative estimate, with true thresholds likely being lower. These audiograms can also be used in conjunction with the field recordings of the pressure component of reef sounds to estimate the potential for sound as an attractant for reef fish larvae.

The audiograms of pre- and post-settlement fish followed similar trends in shape, suggesting that morphological mechanisms of sound detection did not change appreciably (i.e. no hearing specialisations were being added) but the ear may have been getting more sensitive. A clear increase in auditory sensitivity with post-settlement growth has previously been demonstrated behaviourally in one pomacentrid species, *S. partitus* (Kenyon 1996). Although not testing this directly, Kenyon (1996) suggested that the change in sensitivity in *S. partitus* was related to increases in hair cell number, a mechanism that may be operating in *P. nagasakiensis* as well. A clear correlation has been found between increases in auditory sensitivity and hair cell number in the isolated macula neglecta of an elasmobranch (*Raja clavata*) (Corwin 1983). Other studies have found either inconsistent changes in auditory sensitivity with development (Iwashita et al. 1999; Wysocki and Ladich 2001) or no changes in sensitivity with development (Popper 1971; Higgs et al. 2003), leading Higgs et al. (2003) to suggest that considerable improvements in auditory sensitivity might be dependent upon the degree of specialisation for transferring sounds to the ear. *Pomacentrus nagasakiensis* has no known specialisations for sound transfer, so the slight increase in hearing sensitivity seen in this study may be due to changes in hair cell number. If, as expected, hair cell numbers increase with growth, sensitivity changes may be greater later in development as the fish we examined differed in size by only 3–5 mm SL.

Comparison of the audiograms of *P. nagasakiensis* with audiograms of other species shows interesting

parallels and differences. Zebrafish, *Danio rerio*, (Higgs et al. 2002) and goldfish, *Carassius auratus*, (Yan et al. 2000) have greatest hearing sensitivity at 800 Hz, whereas the bicolor damsel, *S. partitus*, was most sensitive at 500 Hz (Kenyon 1996). Increased sensitivity was also seen in the mid-range of frequencies for *P. nagasakiensis*. The hearing of post-settlement and adult stages of the damselfish *A. saxatilis* was also tested by ABR and showed similar peaks in sensitivity as *P. nagasakiensis* at the lower frequencies of 100 and 200 Hz (Egner and Mann 2005). We suspect that in our case study this may be due to detection by both the auditory and mechanosensory (lateral line) system. Our auditory testing apparatus includes an underwater speaker, in contrast to other systems which used a speaker in the air (Kenyon et al. 1998; Yan and Curtsinger 2000). Owing to space limitations our experimental animals were approximately 76 cm from the speaker, well within the nearfield range at both 100 and 200 Hz. Although placing the speaker in the air would have reduced (although not eliminated) the effects of the lateral line stimulation, we chose to use an underwater speaker to more realistically simulate the conditions experienced by the fish in nature.

Audiogram comparisons must be done cautiously. Difference in techniques (i.e. behavioural vs. physiological) can produce dramatically different results. Hearing sensitivity at any given frequency has been found to be 10–30 dB lower, using behavioural measures than using ABR (Gorga et al. 1988; Kenyon et al. 1998). Presumably, this is because the fish sensory system is more sensitive than the ABR electrodes used to measure it. Our ABR results were similar in shape to the behavioural audiogram of *S. partitus* (Kenyon 1996) and are also similar in threshold to similarly sized *S. partitus*. Different methodologies and experimental conditions can also significantly affect the results obtained and make comparison of audiograms difficult. Whereas physiological techniques provide less sensitive results than behavioural testing, they are more amenable for work with larval fish. Behavioural conditioning takes time and the rapid ontogenetic changes at the time of settlement mean that pre-settlement fish quickly and irrevocably become post-settlement fish (McCormick et al. 2002). Also, the results produced in behavioural tests for the same species are often inconsistent with other studies and are not universally applicable (Kenyon et al. 1998).

Traditionally, physiological studies have used pure tones, tone bursts and clicks to test the hearing ability of fish (Kenyon 1996; Kenyon et al. 1998; Wysocki and Ladich 2001; Higgs et al. 2003; Wysocki and Ladich 2003). These sounds, although useful in determining whether a sound can be detected or not, are not sounds that the fish would encounter in their natural environment. In contrast, one ABR study used fish sounds to successfully determine how conspecific sounds are processed by auditory systems of teleost fish (Wysocki and Ladich 2003). Natural coral reef sounds have not previously been used as a stimulus for a physiological test of auditory capabilities. Behavioural studies, however, have found that coral reef fish larvae can hear biologically meaningful sounds (Tolimieri et al. 2000; Leis et al. 2002, 2003; Tolimieri et al. 2004; Leis and Lockett 2005). In the present study, auditory thresholds in response to reef noise were the least sensitive of all the sounds played. This is probably explained by the fact that the reef noise was a raw segment from a recording made over a reef at night, and as such is a composite of a number of frequencies and sound levels.

Field studies clearly demonstrate that the settlement stage coral reef fish larvae are capable of hearing and localising reef sounds (Leis et al. 2003; Simpson et al. 2004; Tolimieri et al. 2004; Leis and Lockett 2005) and our data also show coral reef fish larvae can respond physiologically to such auditory cues. The question remains, however, whether the hearing thresholds obtained here (110 and 150 dB re 1 μPa) are low enough to be biologically significant. Individual reef fish sounds within 1 m of their source can reach 145–160 dB re 1 μPa (McCauley and Cato 2000). If we assume that physiological thresholds underestimate the ability of fish to detect sounds by 20 dB re 1 μPa, as has been shown by several workers (Gorga et al. 1988; Kenyon et al. 1998), then the thresholds presented here will be at least 30–50 dB below source levels of individual fish sounds. With many individuals participating in a synchronized reef chorus (Cato 1978), the output would be even higher than for an individual fish and indeed can reach 20–30 dB above background levels. Thus, the ABR data reported here supports the hypothesis that reef sounds are a detectable cue many kilometres away from the reef.

Olfactory physiology

Both pre- and post-settlement fish were able to detect the odours tested, with the responses to both L-alanine and CW being significantly greater than the background level (Fig. 6). Amino acids are commonly used in olfactory studies of fish (Saglio and Fauconneau 1985; Murphy et al. 2001; Belanger et al. 2005). As the monomers of proteins, amino acids are present in every organism and are a potent food cue for fish (Carr et al. 1977; Ishida and Kobayashi 1992). Thus, olfactory detection of amino acids could be a useful cue for a larva attempting to locate a reef, which represents a concentrated source of amino acids arising from its high density of living organisms. Settlement in a location where there is available food is obviously beneficial, and therefore may be a cue used by larvae to locate a suitable settlement location.

Conditioned water is also a potent settlement cue (Ohman et al. 1998; Leis and Carson-Ewart 2002). Prior studies indicate that olfaction is used by *P. nagasakiensis* (identified as Pomacentrus sp.) to avoid settling with

some *Dascyllus* species (Sweatman 1988). Although there is no direct evidence that *P. nagasakiensis* chooses to settle based on the presence of conspecifics (Leis and Carson-Ewart 2002), the ability to detect other conspecifics in the vicinity would perhaps indicate that a location was a suitable site for settlement.

No significant change in olfactory ability was found between pre- and post-settlement *P. nagasakiensis*. L-alanine produced similar responses in the pre- and post-settlement fish, whereas pre-settlement fish only had a slightly stronger response to CW than their post-settlement counterparts (Fig. 6). The size of the olfactory organ and the quantity of the lamellae have been found to increase with age in certain fishes (Zeiske et al. 1992), but this is unknown for *P. nagasakiensis*. We found no difference between pre- and post-settlement *P. nagasakiensis* but would anticipate differences in older juveniles or adults or in younger larvae. Alternatively, if the olfactory organs of *P. nagasakiensis* are well developed at settlement, no further development may need to take place.

Olfaction is a plausible candidate for a sensory cue that could be used by settlement stage larval reef fish to locate a settlement habitat. Morphologically, peripheral olfactory organs of various settlement stage fish, including apogonids, pomacentrids, blennies and gobies are well developed and are apparently capable of processing olfactory information such as reef odours (Atema et al. 2002). Further, settlement stage apogonids showed a strong preference for lagoon water over oceanic water in behavioural choice chambers (Atema et al. 2002). Olfactory cues from resident conspecifics on coral units influenced the settlement of two damselfish species, *Dascyllus aruanus* and *D. reticulatus* (Sweatman 1988). In anemone fish, imprinting and utilisation of olfactory cues from host anemones by settlement stage larvae has been well documented (Elliot et al. 1995; Arvedlund and Neilson 1996; Arvedlund et al. 1999). Our physiological results corroborate the results found in behavioural studies, directly demonstrating that olfactory stimuli can be detected by marine larvae, thus serving as a potent potential cue for attraction to a reef.

The olfactory abilities of many freshwater fishes have been studied using the EOG technique (Caprio 1978; Michel and Lubomudrov 1995; Sorensen et al. 1995; Valentincic et al. 2000). The present contribution is the first published report of EOGs being successfully carried out on marine fish larvae. Responses were tested rigorously against controls and at no time did a control elicit a response above background level. The responses to introduced odours were significantly greater than the background level recorded during experiments. As all factors were controlled for, and all responses were significantly greater than the background level, we are confident that we recorded EOG responses for marine fish larvae in marine conditions.

The aim of the current study was to identify potential cues for attraction of coral reef larvae to suitable settlement habitats. Our results show that relevant auditory and olfactory cues can be detected by the sensory periphery of reef fish larvae, making both types of information potentially useful. This does not, however, prove that these cues are indeed used by larvae in settlement. Hence, more fine-scale behavioural tests are still necessary. We found little developmental difference in sensitivities of pre- and post-settlement larvae. Seemingly, a high level of sensory competency is reached prior to settlement, and then maintained relatively unchanged for some time. Although we studied sensory abilities on both sides of the ecological transition of settlement, the difference in size of 3–5 mm and in age of two weeks may not be enough to reveal much alteration in sensory abilities. Future studies should examine a wider range of ages and sizes to accurately determine the full trajectory on sensory ontogeny.

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