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RESEARCH ARTICLE

The effects of salinity and temperature on the transparency of the grass shrimp *Palaemonetes pugio*

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SUMMARY

Transparency is an effective form of camouflage, but it must be present throughout the entire volume of an animal to succeed. Certain environmental stressors may cause physiological responses that increase internal light scattering, making tissue less transparent and more conspicuous to predators. We tested this in the transparent grass shrimp, *Palaemonetes pugio*, which is found in shallow estuaries where both salinity and temperature change rapidly because of tidal cycles, evaporation and runoff. Animals originally kept at a salinity of 15 p.p.t. and a temperature of 20°C were placed into solutions with salinities of 0, 15, 25 or 30 p.p.t. and temperatures of 13, 20 or 27°C for 12 h (N=26 for each of 12 treatments). Under the control conditions of 15 p.p.t. at 20°C, the transparency of grass shrimp tails was 54±3% (mean ± s.e.). At higher salinities and at both higher and lower temperatures, transparency dropped significantly (P<0.001, two-way ANOVA), reaching 0.04±0.01% at 30 p.p.t. at 27°C. Confocal microscopy of *P. pugio*'s tail suggested that the observed loss of transparency was due to the pooling of low refractive index hemolymph between the high index muscle fibers, creating many index boundaries that increased light scattering. Analysis of a year-long salinity and temperature record from a North Carolina estuary showed that changes of the order of those found in this study are relatively common, suggesting that *P. pugio* may undergo periods of reduced crypsis, potentially leading to increased predation.

Key words: *Palaemonetes pugio*, camouflage, Crustacea, salinity, temperature, transparency.

INTRODUCTION

Transparency is a highly effective form of camouflage that renders many species nearly invisible (reviewed by Johnsen, 2001). In comparison to other forms of camouflage, however, the effectiveness of transparency is limited in several significant ways. Perhaps the greatest limitation to transparency is that it is profoundly affected by the distribution of densities within a tissue (Johnsen and Widder, 1999). Thus, while other forms of camouflage are mediated by only the surface of the animal, transparency must be maintained throughout the entire volume of an animal's body. In a transparent animal, any fluctuations in density on size scales greater than half a wavelength of light lead to light scattering and an increase in opacity (Benedek, 1971). Thus, physiological responses to environmental stressors that affect density distribution in tissues (e.g. protein precipitation, edemas) can make a previously transparent animal vulnerable to visual predation. While researchers who study gelatinous zooplankton have long been aware that many transparent species become opaque when stressed or moribund (e.g. Hamner, 1984) (reviewed by McFall-Ngai, 1990), this fundamental limitation to transparency has not been quantified. One reason for this is that most transparent species are pelagic, oceanic and fragile, making them unsuitable for laboratory manipulations.

To study the effect of environmental stressors on transparency, we worked with the grass shrimp, *Palaemonetes pugio*, a hardy coastal species that is highly transparent. Found in Atlantic and Gulf coast estuarine systems, *P. pugio* is subject to visual predation from killifish (*Fundulus heteroclitus*), summer flounder (*Paralycthys dentatus*), a diverse array of post-larval decapod crustaceans, and a number of freshwater fish species (Anderson, 1985; Rozas and Hackney, 1984). *Palaemonetes pugio*'s transparency is generally assumed to counter this pressure and, with the exception of a few regions within the thorax, the animal is indeed quite cryptic (Fig. 1A).

The estuaries where *P. pugio* is found experience rapid changes in salinity and temperature due to tides, evaporation and the influx of fresh water from rivers and rain. These fluctuations are generally greatest near the mouths of shallow estuaries, where tides strongly affect water depth. Tides influence water temperature and salinity directly *via* infusion of different water sources and indirectly *via* solar heating and subsequent evaporation. Behavioral responses by *P. pugio* suggest that this animal tries to avoid high temperatures and salinities: it migrates to deeper waters (~15 m) during periods of high temperature (Wood, 1967), though it is also known to migrate to shallow water (<35 cm) when macrophytic cover in deeper waters declines (Ruiz et al., 1993).

In this study, we tested whether ecologically relevant changes in salinity and temperature affect the transparency of *P. pugio* muscle tissue. If temperature and salinity do compromise camouflage in *P. pugio*, it may cause significant increases in grass shrimp predation rates by visual predators in certain ecological contexts.

MATERIALS AND METHODS

Collection and maintenance of *P. pugio*

Specimens of *P. pugio* Holthius from seagrass beds near Palm Bay, FL, USA and Apalachicola Bay, FL, USA (21.41°N, 80.61°W, and 30.11°N, 84.20°W, respectively) were purchased from Sunpet Ltd

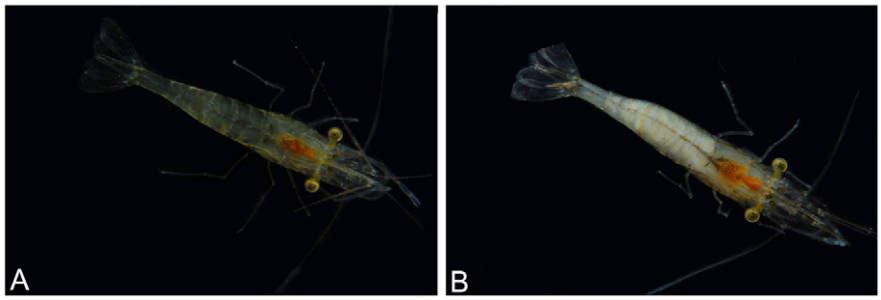

Fig. 1. Visual differences. Comparison of a transparent specimen of *Palaemonetes pugio* (left) with one exposed to 27°C, 25 p.p.t. for 12 h (right).

(Atlanta, GA, USA). Animals were kept in a 20 l aquarium (salinity 13–15 p.p.t.; 20°C, bi-weekly water changes) for the duration of the study. All shrimp used in experiments were kept for less than 10 days. Newly acquired shrimp were acclimated in the holding tank for at least 36 h before experimentation and were fed every 3 days with fish flakes (Community brand, Ocean Nutrition, Salt Lake City, UT, USA). Only individuals that were wider than 1.7 mm at the base of their tail (e.g. between the fourth and fifth abdominal segment) were used in experiments.

Salinity and temperature treatments

Shrimp were placed into individual 8 cm wide, 500 ml jars containing approximately 250 ml of filtered fresh water mixed with varying concentrations of aquarium-grade sea salt (Instant Ocean Inc., Chicago, IL, USA). The treatments were conducted at salinities of 0, 15 (control), 25 and 30 p.p.t., which are consistent with previously observed salinity ranges in the natural habitats of these animals (Wood, 1967). Though previous studies have shown that *P. pugio* have a 96 h LS_{50} of 44 p.p.t. at optimal conditions (that is, exposure to 44 p.p.t. for 96 h is lethal to half the population), they are commonly found in salinities ranging from 2 to 36 p.p.t. (Anderson, 1985). Each salinity treatment was subdivided into three temperature treatments. One-third of the animals were kept at 13°C by partially immersing the jars in a cold-water aquarium, one-third were kept at room temperature (20°C) and one-third were kept inside a Thelco Model 16 incubator (Precision Scientific, Chicago, IL, USA) at 27°C. A total of 26 replicates were used for each treatment. All animals were kept at their assigned temperature and salinity for 12 h, with the exception of 8 replicates, which were incubated for 18 h. However, a *t*-test showed that measurements from these replicates were not significantly different from replicates incubated for 12 h, so all replicates were combined for analysis. Gradual increases of approximately 2 p.p.t. and 2°C per day over the course of 1 week were also conducted, but these measurements were not significantly different from our treatments, and thus were excluded from analysis.

Furthermore, during the re-acclimation time, photographs of the shrimp were taken next to a length standard in order to measure body widths. These photographs were then analyzed using ImageJ software (version 1.42q, NIH, Bethesda, MD, USA). A linear regression showed that body width was significantly correlated with body length (P<0.0001) and that the treatments themselves had no effect on body width.

Measurements of tissue transparency

Spectral measurements were taken immediately after the treatments. These measurements of the transparency of the tail muscle of *P. pugio* were made using a fiber optic-based spectroradiometer (USB2000, Ocean Optics Inc., Dunedin, FL, USA) and an optical apparatus matching that described by Johnsen and Widder (Johnsen and Widder, 1998). Light from a tungsten–halogen source (LS-1, Ocean Optics) was transmitted *via* a 600 μm diameter fiber optic cable to a collimating lens (74-UV, Ocean Optics) that then projected a 4 mm diameter parallel beam through a 4.8×4.8×4.8 cm acrylic cuvette. The portion of the beam that was not absorbed or scattered more than 1.25 deg away from parallel then passed through a 4 mm diameter aperture and was focused *via* a second collimating lens. This focused light was transmitted to the spectrometer *via* a 100 μm diameter fiber optic cable. The cuvette either contained only water (providing a reference measurement) or contained water and a specimen of *P. pugio* mounted so that it lay near the wall of the cuvette closest to the first collimating lens and perpendicular to the beam. All animals were positioned so that the beam passed through the anterior portion of the tail muscle (between the fourth and fifth abdominal segment), chosen because it had the largest cross-sectional area of transparent muscle tissue and minimal potential scattering and absorption from the pigmented organs of the thorax. The spectra were divided by the reference measurement to determine the percentage transparency as a function of wavelength.

Following measurement, specimens were kept at 20°C in fresh water for 24 h or until they regained their transparency, which generally took at least 12 h (preliminary tests showed that the shrimp were equally transparent, active and apparently healthy when kept in either freshwater or 15 p.p.t. seawater). After the shrimp regained their transparency, they were isolated under control conditions (15 p.p.t. at 20°C) for 72 h before returning them to the holding tank to be used for other experiments. Some shrimp were reused in subsequent experiments, separated by several days. On average, each shrimp was used 3 times. Measurements of body size showed that treatment did not affect the optical pathlength though the shrimp muscle.

Normalization of transparency values and data analysis

The transparency spectra were approximately wavelength independent, so we defined an animal's visible transparency to be its average percentage transparency over the 400 to 700 nm interval. Because the shrimp varied somewhat in size, we normalized all transparency measurements to what they would be for a shrimp with a tail thickness of 2.4 mm (the average of the experimental animals). This was done by assuming that light was attenuated exponentially in the tail muscle, i.e.:

$$T = e^{-cx}, \quad (1)$$

where T is the fractional transparency (i.e. 1=100%), c is the attenuation coefficient of the tissue and x is the thickness of the anterior portion of the tail. During the re-acclimation time, x was measured by photographing each animal next to a scale and then analyzing the images in ImageJ (version 1.42q, NIH, Bethesda, MD,

USA). Because $c=-\ln(T)/x$, the normalized fractional transparency T_N of a 2.4 mm thick tail is given by:

$$T_N = e^{-2.4c} = e^{-2.4\left(-\frac{\ln(T)}{x}\right)} = e^{\ln(T)\frac{2.4}{x}} = \left(e^{\ln(T)}\right)^{\frac{2.4}{x}} = T^{\frac{2.4}{x}} . \quad (2)$$

We conducted a two-way ANOVA of this normalized transparency with respect to salinity and temperature. After the ANOVA, we used a *post hoc* Tukey HSD test to compare treatments. The data were not normal and had unequal variances (primarily due to the small variance in transparency for the 30 p.p.t. at 27°C case, where all the animals were essentially opaque). However, it has been shown that ANOVAs, particularly those with a balanced design, are fairly robust against departures from normality and homoscedasticity (Ferguson and Takane, 1989). Nevertheless, to confirm our results, we performed one-way Welch ANOVAs and one-way Kruskal–Wallis tests against salinity and temperature. The first test allows for unequal variances and the second for non-normal data. In all cases, the same approximate *P*-values were obtained. All statistical analyses were performed using JMP 8.0 (SAS Institute, Cary, NC, USA).

Estimation of visibility

Because light is both scattered and absorbed by the tissue, estimating the effect of a loss of transparency on visibility is difficult for most viewing angles. However, it can be estimated for a viewing direction in which the animal is silhouetted by the down-welling light (see Johnsen and Widder, 1998). In this upward viewing direction, an object with a fractional transparency T can be just detected from a distance d (known as the sighting distance) that is given by:

$$d = \frac{\ln\left(\frac{1-T}{C_{\min}}\right)}{c-K_L} = \frac{\ln(1-T)-\ln C_{\min}}{c-K_L} , \quad (3)$$

where $C_{\min}$ is the minimum contrast threshold of the viewer [~0.02 for most fish (Douglas and Hawryshyn, 1990)], c is the beam attenuation coefficient of the water and K_L is the attenuation coefficient of the downward radiance (Johnsen, 2002). While a number of different models relating sighting distance to time-to-capture have been proposed, several commonly used ones assume that time-to-capture t is inversely proportional to the square of the sighting distance (e.g. Aksnes and Utne, 1997). Therefore, the ratio of capture time for a more opaque shrimp after treatment at a given salinity and temperature to that of one in the control conditions of 15 p.p.t. and 20°C is independent of the water's clarity and is given by:

$$\frac{t_{\text{sal,temp}}}{t_{15\text{p.p.t.},20^\circ\text{C}}} = \frac{d^2_{15\text{p.p.t.},20^\circ\text{C}}}{d^2_{\text{sal,temp}}} = \frac{\left(\ln\left(1-T_{15\text{p.p.t.},20^\circ\text{C}}\right)-\ln C_{\min}\right)^2}{\left(\ln\left(1-T_{\text{sal,temp}}\right)-\ln C_{\min}\right)^2} . \quad (4)$$

Values of this ratio were calculated for all the measured transparencies for minimum contrast thresholds of 0.02 and 0.1, values typical for fish in daylight and twilight (Douglas and Hawryshyn, 1990).

Tissue fixation and microscopy

Sample animals from the various treatments were anesthetized in 1% solutions of Tricaine methanesulfonate (Finquel, Argent Chemical Laboratories, Redmond, WA, USA) for 20–30 min, using a procedure similar to that used by Schwartz (Schwartz, 1966). The shrimp were then fixed in a 4% formaldehyde solution buffered in 20 p.p.t. seawater for 48 h at approximately 20°C and then stored in 70% ethanol. Fixed shrimp were sectioned using a Miles Tissue Tek II cryostat microtome and were stained following a procedure described previously (Speiser and Johnsen, 2008). Anti-tropomyosin and anti-actinin were used as the primary antibodies and Alexa Fluor 488 and Alexa Fluor 568, respectively, were employed as secondary antibodies (Invitrogen, Carlsbad, CA, USA). Tropomyosin and actinin are two abundant muscular proteins that, we believed, could show the organization of the tissue ultrastructure. Multiple proteins were used to provide better contrast. Sections were imaged using a Zeiss 510 LSM confocal microscope with a ×10 objective and were illuminated by 488 and 568 nm wavelength lasers. Images from the confocal were processed using Zeiss LSM 510 software (version 4.2, Carl Zeiss Inc., Oberkochen, Germany).

In situ salinity and temperature measurements

Salinity and temperature records were obtained from Dr John Fear at the North Carolina National Estuarine Research Reserve. Briefly, measurements were taken at Middle Marsh, NC (34°42″N, 76°32″W) from the period of January 2003 to December 2003 using a YSI 6600EDS non-vented sonde (YSI Inc., Yellow Springs, OH, USA). Before deployment, calibration and maintenance were performed following the manufacturer's instructions (YSI Manual addendum 7/94, sections 3, 4 and 7). The conductivity standard was obtained from the manufacturer. The sondes were kept in a wet towel before deployment. During deployment, the weather conditions and tide stage were recorded. The instrument was deployed approximately 15 cm above the bottom and chained to an observation post. Temperature and salinity measurements were taken every 30 min during the deployment period. At the end of the deployment period, the sondes were collected and transported back wrapped in a wet towel. The calibration drift was checked by comparing data readings in calibration standards.

RESULTS

Temperature and salinity levels significantly affected normalized tissue transparency in the grass shrimp *P. pugio* (Figs 1, 2; Tables 1, 2). Combining salinity treatments for all three temperatures, light transmittance through tail tissue was highest in the 20°C control treatment (45±2%, mean ± s.e.) and significantly lower at 13°C (22±1%) and 27°C (14±2%). Combining temperature treatments for all three salinities, light transmittance was highest at 0 p.p.t. (43±2%) and steadily declined with increasing salinity (34±2% at 15 p.p.t.; 23±2% at 25 p.p.t.; 8.9±1.5% at 30 p.p.t.). All treatments were significantly different from each other, with the exception of 0 and 15 p.p.t., which were not different from each other, but were different from all other treatments (Table 2).

Changes in salinity also affected temperature tolerances. For example, at a temperature of 27°C a shrimp at 30 p.p.t. was less transparent than a shrimp at 15 p.p.t. Shrimp exposed to 13°C and 27°C generally had lower transparencies than shrimp at 20°C at any salinity (i.e. the minimum of the 20°C curve is above almost all points on the 13°C and 27°C curves). The interaction of salinity and temperature was also significant ($F_{6,310}$=4.25, P=0.0004). That is, at 13°C and 27°C, the effects of increasing salinity were more pronounced than they were at 20°C. The analysis also showed that the 13°C treatments had significantly higher light transmittance than the 27°C treatments at the 15 and 25 p.p.t. salinities, but were not different at the extremes of 0 and 30 p.p.t. We also observed that the opaque shrimp did not exhibit any drastic changes in behavior,

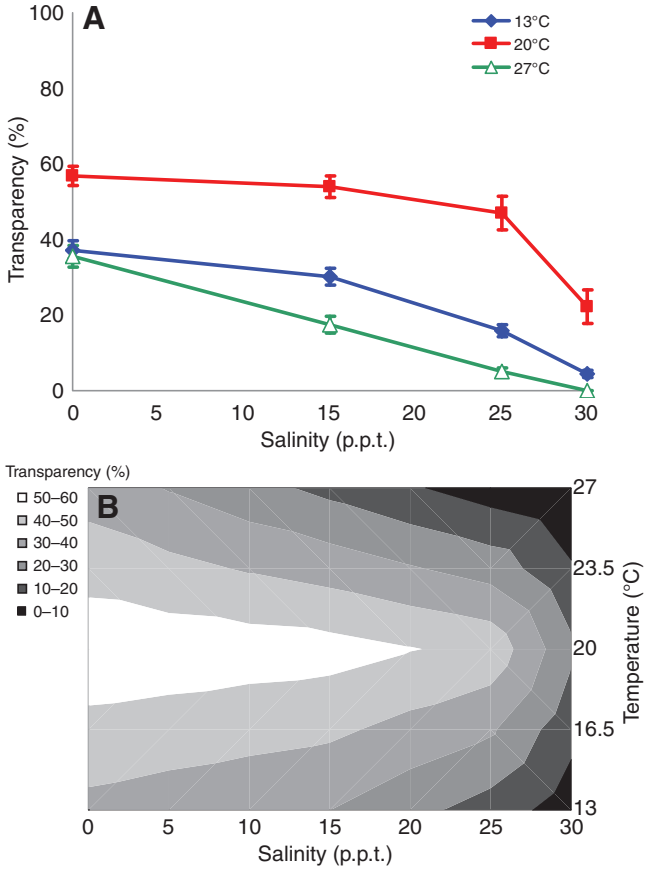

Fig. 2. Treatment effects. (A) Light transmission through 2.4 mm of shrimp tail muscle as a function of salinity at 13, 20 and 27°C. Error bars are standard error. (B) Linear interpolation of light transmission (%) through 2.4 mm tissue based on data from the 12 experimental conditions.

but seemed to move slower and had slower reaction times when being netted.

The observed changes in transparency had a significant effect on the estimated time-to-capture (Fig. 3). Under daylight conditions (C_{min}=0.02), the animals that were nearly opaque had a sighting distance that was approximately 25% longer than that of those in the control condition, resulting in a time to capture that was approximately 64% that of the control. Under twilight conditions, (C_{min}=0.1) the animals that were nearly opaque had a sighting distance that was approximately 50% longer than that of those in the control condition, resulting in a time to capture that was approximately 44% that of the control.

Salinity and temperature records from Middle Marsh, NC, showed that *in situ* temperatures ranged from 2 to 33°C and salinities ranged from 12 to 30 p.p.t. (Fig. 4). Fourier analyses of the records showed cyclic variations with cycle lengths of one solar day, one lunar day (~24.8 h) and various fractions of the lunar day. Salinity in particular had a significant cycle with a periodicity of half a lunar day (one tidal cycle). Temperatures and salinities changed by as much as 8°C and 11 p.p.t. within a 24 h period and by as much as 11°C and 16 p.p.t. over the course of a week (Fig. 5). While temperatures and salinities changed less over 1 h periods, salinity still changed by at least 7 p.p.t. over 40 different 1 h periods in the year.

Confocal microscopy showed clear differences between transparent muscle tissue and opaque tissue (Fig. 6). In the opaque tissue, gaps, which appear black, were seen between muscle fibers, whereas in transparent tissue, these gaps were reduced or non-existent. Opaque tissues (Fig. 6B,D) also showed disorganization of the muscle fibers, leading to the appearance of clusters of muscle fibers. In contrast, transparent tissues (Fig. 6A,C) showed a comparatively more uniform organization of fibers.

Table 1. ANOVA table for the one-way ANOVAs for temperature (N=78; top) and salinity (N=104; middle), and two-way ANOVA (N=312; bottom) of light transmission through 2.4 mm tissue with respect to salinity and temperature

Source/treatment	d.f.	F-ratio/χ^2 value	P
Temperature data			
ANOVA	2	75.66	<0.0001
Kruskal–Wallis	2	95.81	<0.0001
Welch ANOVA	2	61.93	<0.0001
Salinity data			
ANOVA	3	47.20	<0.0001
Kruskal–Wallis	3	122.50	<0.0001
Welch ANOVA	3	60.70	<0.0001
Two-way ANOVA data			
Two-way ANOVA	11	55.71	<0.0001
Temperature	2	150.02	<0.0001
Salinity	3	95.52	<0.0001
Salinity×temperature	6	4.26	0.0004

F-ratios are given for ANOVA results; χ^2 values are given for Kruskal–Wallis results.

DISCUSSION

In situ variation in temperature and salinity

Estuaries are highly dynamic eco-systems. Our sample record showed that daily changes in temperature and salinity are generally less than 4°C and 4 p.p.t., but can be as high as 8°C and 11 p.p.t. Weekly changes are correspondingly higher, reaching 11°C and 16 p.p.t. Even over 1 h, salinity changes can be as high as 10 p.p.t. Our experiment consisted of 7°C and 10 p.p.t. changes over a 12 h period, which mirrored the upper limits of the changes these animals face in their natural environment. Our experimental treatment was introduced instantaneously, which may have caused us to overestimate the effects of temperature and salinity on transparency. However, as discussed in Materials and methods, preliminary experiments using slow changes of salinity or temperature gave equivalent results. We also recognize that the environmental data collected from Middle Marsh may not reflect the exact range of conditions experienced by the particular shrimp used in this study. However, *P. pugio* is also found at this location, and we are confident that our salinity and temperature data are relatively typical of the shallow estuarine systems that these animals inhabit.

Salinity and temperature effects

Changing salinity and temperature simultaneously reduced transparency in *P. pugio* more than through changing just one of

Table 2. *Post hoc* Tukey HSD test results of the differences between the treatment interactions

	0 p.p.t.	15 p.p.t.	25 p.p.t.	30 p.p.t.
13°C	B,C	C,D	E,F	F,G
20°C	A	A	A,B	D,E
27°C	B,C	E,F	F,G	G

Treatments not significantly different from each other (P>0.05) are assigned the same letter. Means are presented in descending order from A to G.

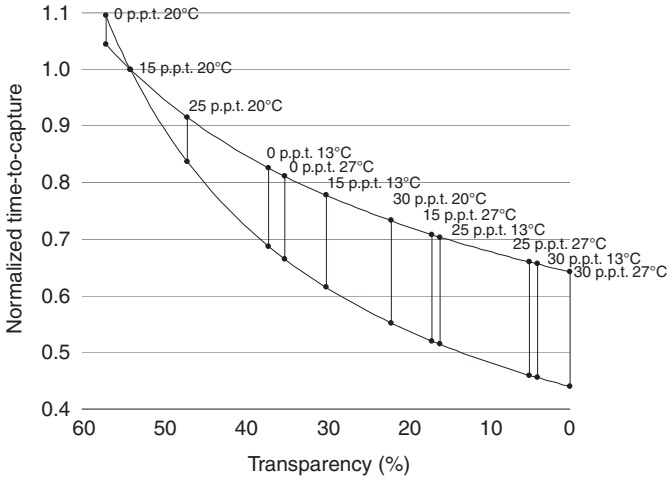

Fig. 3. Effects on time-to-capture. Time-to-capture (normalized by that for a shrimp under control conditions) *vs* percentage transparency of the animal, with the specific values for the various conditions indicated. The upper line assumes the viewer has a contrast threshold of 0.02. The lower line assumes a contrast threshold of 0.1. The vertical lines between the two curves are for clarification purposes only.

the parameters. It is generally accepted that optimal conditions for one variable generally correspond to maximal tolerance to changes of another (Vernberg and Piyatiyaratitivorakul, 1998). That is, at optimal temperatures, grass shrimp tolerate the widest range of salinities, and *vice versa*.

Our results suggest that temperature affects tissue transparency more than salinity does. For example, shrimp at 20°C were significantly more transparent than shrimp at 13 and 27°C across all salinities. However, similarly lowering salinity from 15 to 0 p.p.t. did not significantly reduce transparency at any temperature. In fact, in certain cases, lowering salinity from 15 to 0 p.p.t. increased transparency, though the change was not statistically significant. Although changes in salinity and temperature are not analogous (that is, a change of 1 p.p.t. is not the same as a change of 1°C), the effective temperature and salinity ranges (13–27°C and 0–30 p.p.t.) were similar to the ranges found in our sample record. We assume that the range of ~0–15 p.p.t. is ideal for transparency in these grass shrimp, as they were acclimated to these conditions and because salinities over this range did not reduce transparency. At higher salinities, transparency dropped by at least 50% (at 20°C, 30 p.p.t.) and up to 84% (at 27°C, 25 p.p.t.).

The physical basis for the loss of transparency

For a substance to be transparent, it must have a uniform refractive index over all size scales greater than one-half a wavelength of light (~275 nm for 550 nm green light). Any disruption of this uniformity leads to opacity (Benedek et al., 1979). The pooling hemolymph in the *P. pugio* tail (Fig. 6) creates regions that have a low refractive index relative to the surrounding muscle fibers. While we did not directly measure these different materials, the refractive index of muscle is generally ~1.5 and that of extracellular fluid is ~1.35 (reviewed by Johnsen and Widder, 1999). Therefore, at each interface between the hemolymph and the muscle, at least 0.3% of the incident light is scattered, based on Fresnel equations for back-reflection and assuming perpendicular incidence of the light $[=(1.5–1.35)^2/(1.5+1.35)^2]$ (Hecht, 1998). Although this seems small, there are a large number of these interfaces along a 2.4 mm pathlength within the tail muscle, and the total amount of light transmitted is 0.997 (1–0.3%) to the power of this number. For example, a hemolymph pool every 20 µm (a conservative estimate), would create 120 interfaces and a 30% loss of transparency.

Physiological responses to salinity and temperature change

Sudden increases in salinity and temperature may alter homeostatic mechanisms in *P. pugio* and lead to a breakdown of circulatory processes. Exactly how these mechanisms are affected by sudden changes in temperature and salinity and how disruption of these mechanisms can cause opacity is unknown. We propose that there may be multiple mechanisms that cause opacity *via* pooling of excess fluid in the extracellular space between muscle fibers.

One mechanism could be based on a reduction in respiratory efficiency. Indeed, studies have shown that abrupt increases in salinity and temperature can lower respiratory efficiency in *P. pugio*

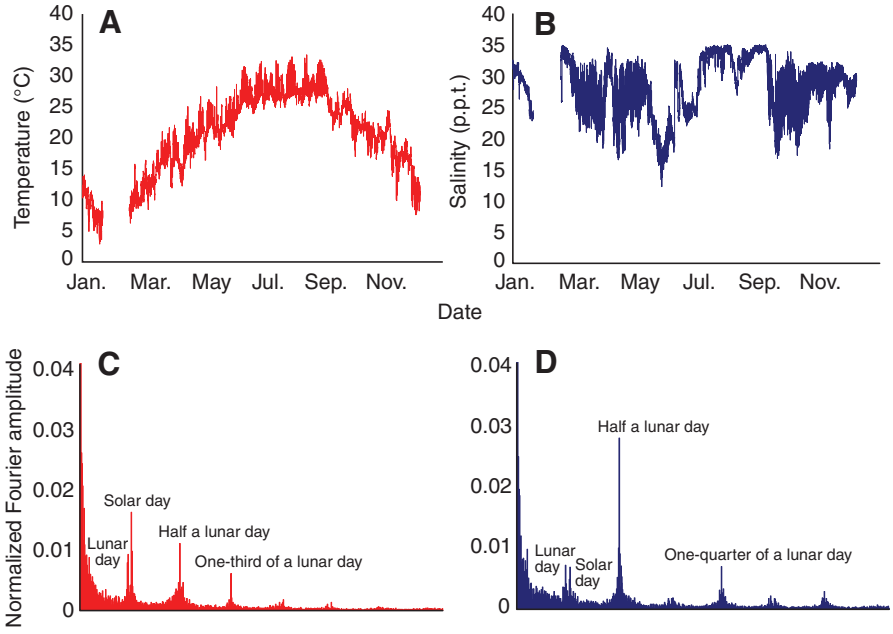

Fig. 4. Salinity and temperature data. (A) Temperature and (B) salinity in Middle Marsh, NC (34.7°N, 76.6°W), from January to December, 2003. Data are missing for the period between 21 January and 16 February. (C) Fourier decomposition of the temperature record. (D) Fourier decomposition of the salinity record. Each graph is the mean of the decompositions of three non-overlapping time periods, each totaling ~85 days (4096 data points; the limit of the software), and thus does not give an accurate Fourier amplitude for cycles with periods significantly longer than a month. Fourier amplitude is normalized in both cases by the zero order to facilitate comparison between C and D. The major cycles have periodicities related to the solar and lunar (i.e. tidal) day.

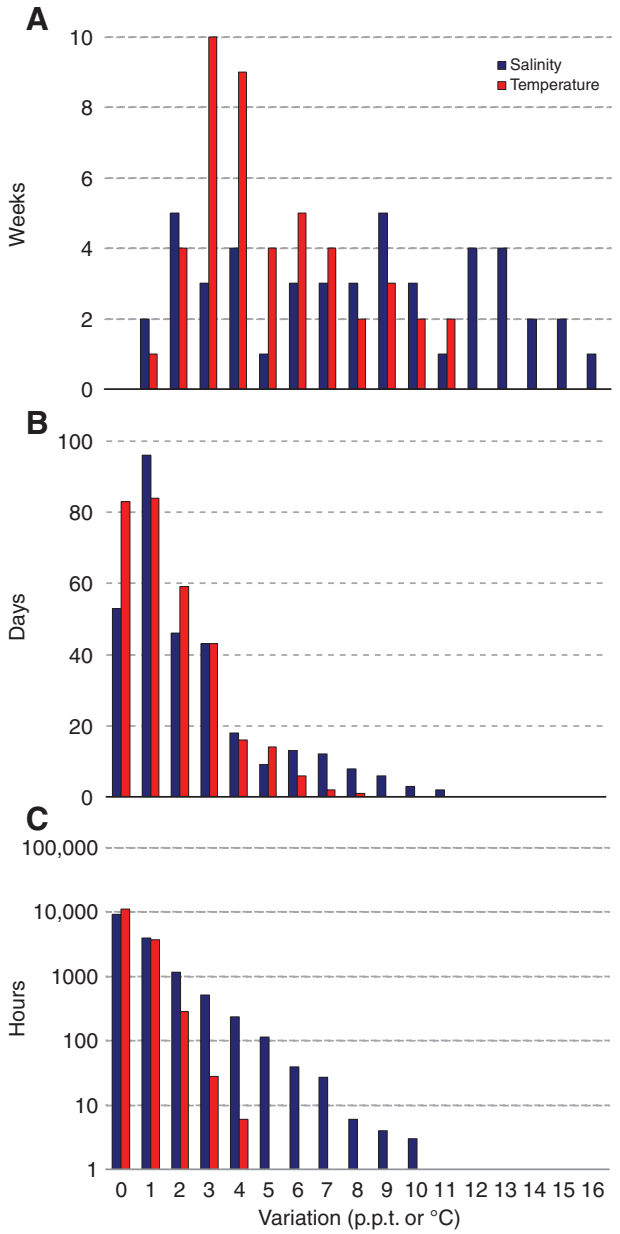

Fig. 5. Histograms of salinity and temperature data. Histograms of absolute changes in temperature and salinity over each week (A), day (B) and hour (C). The 1 h histogram is given in log form because of the large number of data points and high variation.

(Lucu et al., 1977; Von Oertzen, 1984). A decrease in respiratory efficiency increases intramuscular CO_2 levels, leading to low pH in the muscle fibers and alkalosis in the extracellular space (Whiteley et al., 2001). This respiratory alkalosis leads to the accumulation of fluid between muscle fibers (Wheatly, 1985), resulting in increased light scattering.

Rapid changes in temperature have been shown to impact heart rates in all crustaceans (Spaargaren and Achituv, 1977). Therefore, the pooling of hemolymph may be due to a reduction of heart pumping efficiency in *P. pugio*. The pressure difference between the heart and the pericardial sinus is decreased as a result of the reduction of cardiac contraction pressure (Guadagnoli et al., 2007). As the pressure difference facilitates the filling of the ventricle, a lower pressure reduces filling efficiency and increases hemolymph pooling within the intramuscular space.

A third possible mechanism could be the effects of hypertonicity on the muscle fibers themselves. Previous studies have shown that changes in both temperature and salinity can affect total water content in *P. pugio* (Vernberg and Piyatiyaratitivorakul, 1998). Studies in *Drosophila melanogaster* have shown that in hypertonic solutions, water from tissues drains into the hemolymph (Folk et al., 2003). Mat and Potts have also shown that at very high salinities, muscle extracellular space increases and water content of cells decreases (Mat and Potts, 1985). This reduction in volume of muscle fibers and increased space between fibers may lead to disorganization of the muscular tissue ultrastructure.

Finally, crustacean hemocyanin has a lower oxygen affinity at temperatures above 25°C and below 15°C (Burnett et al., 1988). Studies on the rockpool prawn, *Palaemon elegans*, have shown that hemocyanin oxygen affinity decreases by ~80 and ~45% at 25 and 5°C, respectively, relative to oxygen affinity at 15°C (Morris et al., 1985). Lower oxygen affinities may further contribute to respiratory inefficiency, worsening alkalosis and leading to increased fluid accumulation.

Effects of increases *vs* decreases in salinity

Salinity also significantly altered transparency, but only at levels above 15 p.p.t. This is unsurprising, given *P. pugio* can be found far upriver in oligohaline systems, where salinities drop to nearly zero (Kneib, 1987), as well as in areas where tidal activity can change salinities rapidly. Sudden increases of salinity may have different effects *P. pugio* than sudden decreases. Studies on *P. elegans* have shown that a sudden 9 p.p.t. increase in salinity causes a 120% increase in oxygen consumption (i.e. metabolic) rates for 3 h after the increase; whereas a sudden decrease of the same magnitude does not significantly affect oxygen consumption rates (Von Oertzen, 1984). Increased oxygen need has been shown to lead to high cardiac output and stroke volume in crustaceans (DeWachter and McMahon, 1996), both of which can cause and compound hemolymph accumulation in the extramuscular tissue, leading to opacity.

Effects of chromatophores on transparency

Palaemonetes pugio also has chromatophores along the length of its body. Studies have shown that chromatophore density alters in response to changes in light intensity or substrate (Fingerman and Tinkle, 1956). As the killifish *F. heteroclitus* is generally a daytime feeder, changes in both chromatophore density and tissue opacity may combine to further affect *P. pugio*'s visibility to predators. The shrimp's chromatophores are white, red and brown, but their effects are generally small compared with the whole-body opacity we observed in this study (Fingerman and Tinkle, 1956). We believe that in transparent shrimp, chromatophores will have minimal effects on transparency, even if activated. However, we recognize that future studies are needed to ascertain the effects of chromatophores on visibility.

Ecological implications

Changes in transparency in *P. pugio* increase its sighting distance and, using typical foraging models, reduce its time-to-capture, particularly under low light conditions when the contrast thresholds of potential predators are higher. Thus, these optical changes may affect the predation pressure that this species faces. If there is increased predation on these shrimp by fishes, the nutrient dynamics

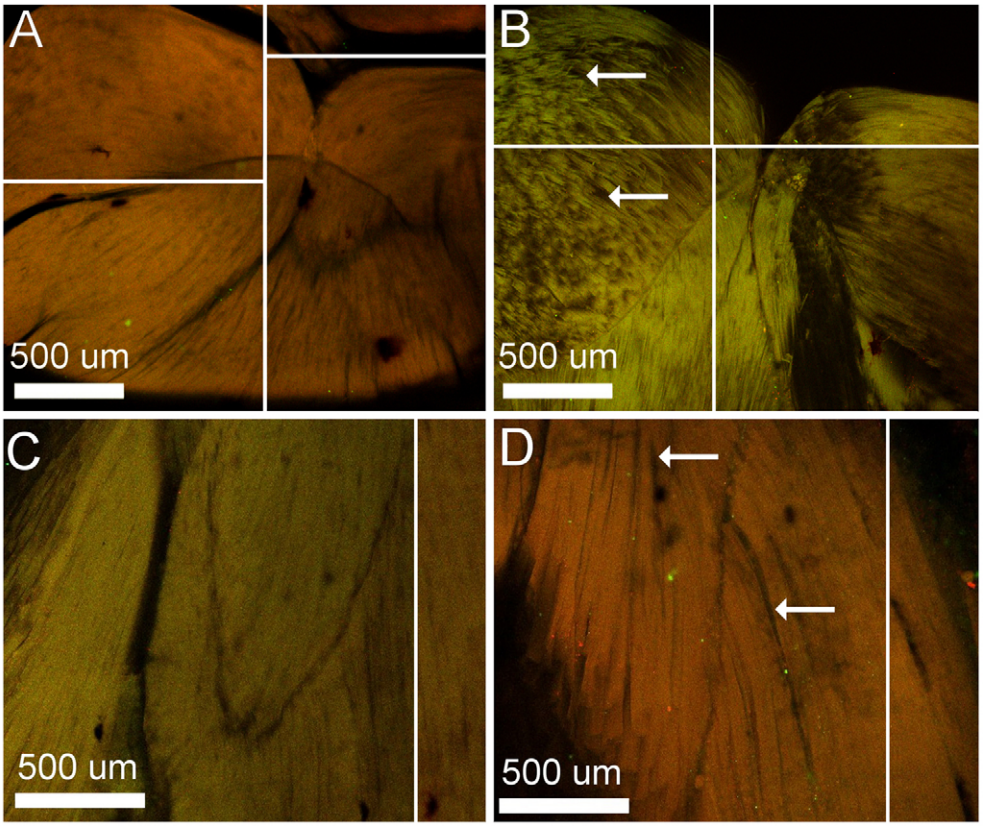

Fig. 6. Confocal microscopy images. Tail muscle of *P. pugio* stained for tropomyosin and actinin and imaged using a confocal microscope with 488 and 568 nm lasers. (A) Transverse section of a transparent shrimp. (B) Transverse section of an opaque (normalized fractal transparency, T_N<5%) shrimp, with arrows marking the areas of greatest disorganization. (C) Parasagittal section of a transparent shrimp. (D) Parasagittal section of an opaque (T_N<5%) shrimp, with arrows marking the areas of the largest 'hemolymph pools'. Note that each panel is a mosaic of at least two images (delineated by white lines) to increase the field of view. The colors of each panel do not signify any differences between them, and are only intended to provide the best contrast in each panel.

of estuaries may be affected. *Palaemonetes pugio* plays an important role in nutrient recycling in estuarine systems, consuming approximately 1000 kcal m^{-2} (~4184 kJ m^{-2}) of decaying organic matter per year and contributing ~90% of the suspended nitrates and phosphates that are taken up by plants (Welsh, 1975). Welsh has also shown that *P. pugio* has symbiotic bacteria in its hindgut that consume the undigested plant matter in its fecal pellets, contributing more nutrients that can be captured and ingested by filter feeders (Welsh, 1975). This leads to a higher nutrient recycling efficiency than achieved *via* grazing by amphipods and meiofauna. *Palaemonetes pugio* has also been shown to consume epiphytes on estuarial macrophytes, specifically *Zostera marina* and *Spartina* spp. (Anderson, 1985). Morgan showed that, though they are not predators, some larger shrimp are able to consume small invertebrates, such as mysids (Morgan, 1980). Finally, consumption by *P. pugio* may help regulate meiofauna populations; the exclusion of *P. pugio* significantly increases populations of meiofauna and epiphytes on *Spartina* plants (Gregg and Fleeger, 1998).

There are a number of other transparent species in estuarine systems, including hydromedusae, ctenophores, larval fish and various crustaceans. Anecdotal evidence suggests that physiological stress reduces their transparency as well. Thus, the dynamism of estuarine systems may have a largely unexplored effect on visual predation and trophic dynamics.

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