

# Heart and ventilatory measures in crayfish during environmental disturbances and social interactions

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## Abstract

Most animals assess the environment in which they live and alter their behavior according to various stimuli. When the animal does not make significant behavioral changes, as measured by bodily movements, the animal may be characterized as unresponsive to a given stimulus. This study demonstrates that when behavioral movements of crayfish cannot be observed, physiological measures of heart rate (HR) and ventilatory rate (VR) show dramatic changes in response to defined sensory stimuli. In the majority of cases, upon anticipation of a social interaction with another crayfish both HR and VR will increase. During an agonistic encounter between two crayfish, the level of HR and VR correlate with the intensity of the interaction. Such rapid responses in cardiac and respiratory systems to environmental disturbances and anticipation of a social interaction suggest an autonomic-like regulation associated with fear, flight or fight. Since behavioral observations do not allow an internal status to be readily assessed, we suggest that HR and VR may serve as a useful bioindex in crustaceans to their internal drive or possibly an awareness level to environmental cues. © 2002 Elsevier Science Inc. All rights reserved.

*Keywords:* Heart rate; Ventilation; Crayfish; Crustaceans; Social status

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## 1. Introduction

Fear, fight or flight responses that are commonly associated with vertebrates are the fundamental physiological responses that allow an animal a competitive edge for survival in the face of impending danger (Carpenter, 1976; Nicholls et al.,

2001). The physiological responses in conjunction with the autonomic nervous system are so well evolved in vertebrates that it would not be surprising to imagine that complex invertebrates possess an analogous response system (Zavarzin, 1941). Highly developed invertebrates also need the same rapid cardiovascular and respiratory regulation to be primed for 'fight or flight' when the need arises. The associated neural and humoral control of bodily function by the autonomic nervous system has likely evolved to regulate basic survival strategy in vertebrates as well as in

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invertebrates. In all animals, the ability to escape predation or to be alert to subtle changes in the environment related with autonomic control is associated with the complex ability to integrate sensory information as well as motor output to target tissues. Autonomic control of the cardiovascular and respiratory systems can regulate the availability of potential oxygen and nutrient needs of the tissue, while at the same time not appearing to cause any external behavioral change that could be assessed by others around it as to its own state of internal readiness. Unfortunately, this makes it hard for behavioral biologist to assess an animal's internal state by direct bodily observations.

Previous investigations (Larimer and Tindel, 1966) in crayfish had indicated that they were not responsive to the presence of other animals because HR did not change substantially, but more recent studies (Listerman et al., 2000; Li et al., 2000) had shown that the presence of another crayfish detected by vision or by olfaction, from the addition of crayfish conditioned water, readily causes alterations in HR. In addition, in hermit crabs it is known that HR is affected during agonistic interactions (Cuadras, 1979, 1980). Alterations in VR were not assessed in these studies but have been examined by Wilkens (1976), McMahon and Wilkens (1983), and Burmistrov and Shuranova (1996). These studies had demonstrated that VR represents a functional state that correlates to environmental changes, thus indicating that like HR, VR allows one to index an autonomic state of the animal. As McMahon (1995) had mentioned, the responses in crustaceans to environmental disturbances are similar to those of vertebrates that are regulated by autonomic control. The purpose of our study is to determine if simultaneous measures of HR and VR may serve as good biological indices to assess if crayfish are sensitive to alterations in its environment during which time behavioral observation would not be as favorable for assessment. In addition, we wanted to know if measures of HR and VR would provide insight into the function of the autonomic state of crayfish prior and during agonistic encounters. Our working hypothesis is that when two crayfish see each other and visually display postural signals, that indicate a pending aggressive encounter, there would be physiological processes that prepare the animal for the

potential body and cellular stress, such as increasing ventilation and cardiac performance.

Part of this work was previously presented in abstract form (Schapker et al., 2000, 2002).

## 2. Methods

### 2.1. Animals

*Procambarus clarkii* (6–10 cm body length) were obtained from a commercial supplier, Atchafalaya Biological Supply Co. (Raceland, LA). The animals were housed in an aquatic facility within our regulated-temperature laboratory (13–16 °C). They were kept in individual tanks and fed fish food pellets weekly until the time of experimentation.

### 2.2. Recording procedures

The same procedures were used as described in an earlier study for obtaining heart rates (Listerman et al., 2000). In brief, two insulated iridium/platinum wires (diameter 0.005 inches and with the coating 0.008 inches; A-M systems, Inc., Carlsburg, WA) were placed under the dorsal carapace directly over the heart to record the heart rate (HR). These two wires were placed to span the heart in a rostral–caudal arrangement to insure an impedance measure during each contraction. A second pair of wires was placed under the cuticle in the rostral area of the gill chamber (i.e. prebranchial chamber) to monitor the ventilatory rate (VR). Wires were inserted through holes drilled in the carapace and cemented in place with cyanoacrylate ester and accelerator (HobbyTown USA, Lexington, KY). The placement of the recording leads are shown in Fig. 1. The use of this rapid drying glue reduced handling stress of the animals which is known to have an effect on HR (Wilkens et al., 1985; Listerman et al., 2000). All the recording experiments were conducted 2–3 days after the initial wiring of the animals.

Impedance detectors (UFI, model 2991) were used, which allowed HR and VR to be monitored as a measure of dynamic resistance. These signals were recorded to a VHS tape (Vetter, 400) and on-line to a PowerMac 9500 via a MacLab/4s interface (ADInstruments). All events were measured and calibrated with the MacLab Chart soft-

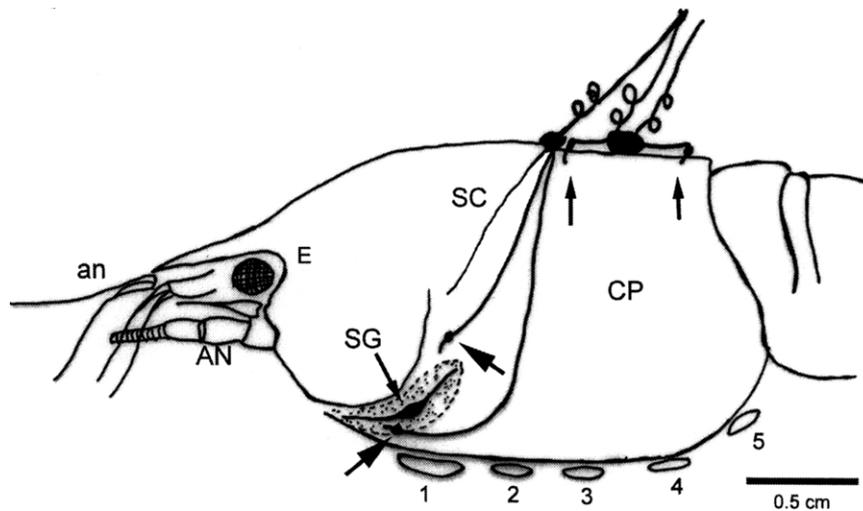


Fig. 1. Schematic representation for the placement of the recording leads for monitoring the heart and ventilatory rates from a crayfish (*Procambarus clarkii*). On the lateral side (large arrows), two leads span the scaphognathite (SG) (i.e. prebranchial chamber-outlined by the dotted line) to monitor any change in the dynamic resistance, which is used as a measure of ventilation. On the dorsal carapace, two other leads (smaller arrows) span the rostral-caudal axis of the heart to monitor heart rate. (See Listerman et al., 2000 for dorsal view of carapace and electrode placement). E-eye, AN-antenna, an-antennule, CP-carapace, SC-sutura cervicalis; thoracal legs (1–5) are cut; mouth appendages are not shown.

were version 3.5.6 (ADInstruments, Australia) with an acquisition rate set at 4 kHz. The instantaneous HR and VR were determined as the inverse of the time period between successive events selected by the window discriminator of the chart software. The values were then converted to beats per minute (BPM).

### 2.3. Behavior

To test the animal's sensitivity to particular environmental alterations, we first examined changes in HR and VR to exposure of white light after the animals were dark-adapted. Minor disturbances of the water in front of a stationary individual, consisted of a drop of water, as a slight mechanical disturbance to their environment. To deliver a stronger intensity of a stimulus, a pebble (~0.15 g) was dropped into the animals tank from 100 cm above the surface of the water (Listerman et al., 2000). To assess if HR and VR were also altered due to social cues from conspecific crayfish, crayfish were paired within an environment so as to promote social contact. For the paired interactions among males, the partners were placed into a divided observation tank. Baseline recordings were obtained during this time of approximately 40 min which is sufficient to determine basal conditions. The divider was

lifted in order for the interactions to proceed. Since all recordings of electrophysiological responses were displayed on an oscilloscope and the behavior on video, they were captured together. A synchronized timer which was captured on video served as common reference. Therefore, the behaviors could be precisely synchronized with the HR and VR.

Dim white lighting was used during these experimental conditions. The individual behaviors as well as the interactions of crayfish were taken using a CCD camera (Toshiba, model IK-537A) fitted with a zoom lens (Pentax TV, zoom 8–48 mm) and a video cassette recorder (Panasonic, time lapse SVHS, model AG6T20). In the environmental disturbance studies a light was supplied by four neon lights (40 W), placed behind a thin sheet of opaque plastic to diffuse the light, 2 m away from the observation tank.

## 3. Results

### 3.1. Environmental disturbances

It is known from earlier studies (Larimer, 1964) that direct tactile stimuli can induce an increase in VR without significant changes in HR. In addition, while the VR can rapidly decrease or in-

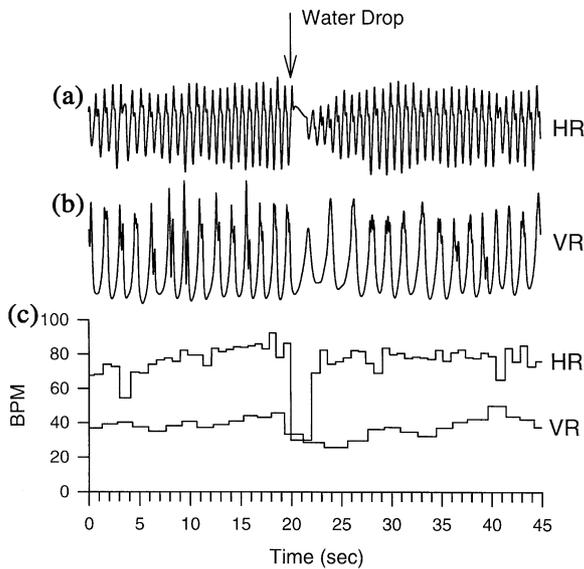


Fig. 2. Representative recordings of heart rate (HR) and ventilatory rate (VR) prior to and after dropping a small drop of water in the aquarium. Note that both the HR (a) and VR (b) markedly decreased for a few beats. At a later time the rates increase (see Fig. 3). The running average of the instantaneous measure of HR and VR in beats per minute (BPM) illustrates the degree of change associated with the single stimulus (c).

crease, the waveform can also change in amplitude (Fig. 2). Upon addition of a drop of water into the aquarium, both HR and VR may rapidly slow down for a few beats before increasing to a higher rate than observed during a resting state of the animal. The initial responses are illustrated in Fig. 2 for both HR and VR. The rate of change for HR and VR are shown as a running mean, in beats per minute (BPM), over time (Fig. 2c). The alterations in VR and HR responses depicted in Fig. 2 occurred in the absence of any observable behavioral modifications. This type of HR and VR response without a behavioral modification is typical for such minor stimuli.

When a 24-h dark-adapted crayfish is exposed to overhead light, a relatively rapid (< 5 s) response is induced in both the HR and VR over basal conditions (Fig. 3). Fig. 3 shows mean responses of HR (Fig. 3a) and VR (Fig. 3b) to the light stimulus for each second. The responses can be defined as consisting of an initial and delayed component, however, the amount of change for each component varied considerable from crayfish to crayfish. Our findings show that the initial

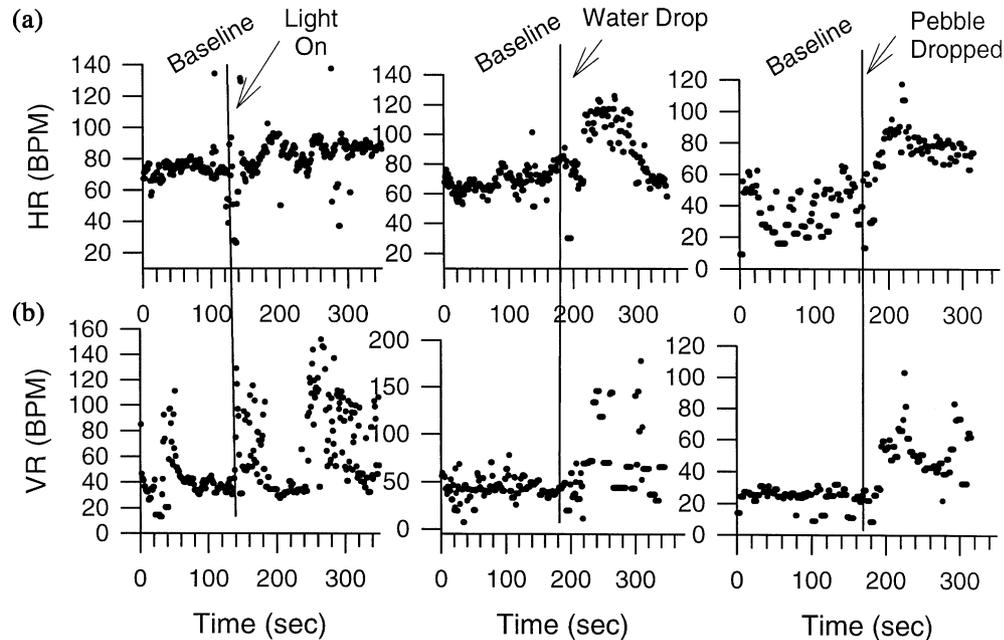


Fig. 3. Changes in HR (a) and VR (b) correlated to the three types of physical disturbances (light, a drop of water, a pebble dropped in the aquarium) that were used to assess responsiveness of the crayfish. The presence of light, after being dark adapted for 24 h, results in an increase in both HR and VR prior to the initiation of bodily movement, at which time the recordings were terminated (left column). A drop of water falling from 100 cm to the surface of the water in the aquarium and in front of the animal, causes a rapid decrease following by an increase in HR and VR (middle column). A more pronounced response occurred when a 0.15-g pebble was dropped from the same height (right column).

short component of all these responses is mostly inhibitory followed by an excitatory response. In the delayed phase a substantial increase in HR or VR occurred in five out of five crayfish ( $n = 5$ ,  $P < 0.05$ , Wilcoxon test) (Fig. 4), but in two crayfish one or the other indices changed but not in unison (Fig. 4). In addition, usually the animal would change its bodily position (turned toward the stimulus while others stood up on their walking legs and moved forward and backwards without any obvious directional association with the stimulus) shortly after the heightening of the cardiovascular or respiratory response. In some cases, a change in VR occurred slightly before the change in HR, however, both rates increased before a animal would undergo ambulatory activity. In another series of experiments conducted

on the same individuals but after 5 h of not being disturbed, a drop (100  $\mu$ l) of water was dropped from a distance 100 cm above the surface of water within the aquarium. It was apparent from responses of the HR and VR that the 5-h rest period was sufficient to insure that the animals regained a basal level before being exposed to a subsequent environmental stimulus. Four out of five crayfish had substantial changes in both HR and VR, where as one out of five showed only a slight enhancement in its HR and VR (Fig. 4). Representative measures, in the same crayfish as previously indicated for the response to light, are shown in the middle column of Fig. 3. The following day (18 h after the water drop exposure) a stronger stimulus was given to the animals. This time, a small pebble was dropped from the same

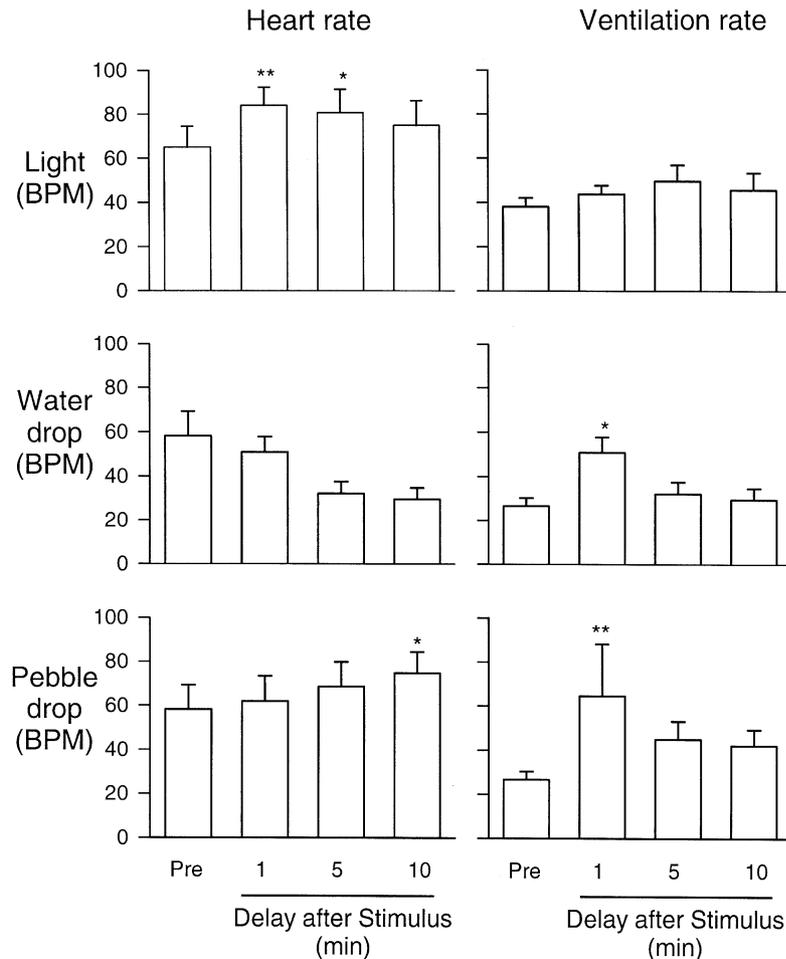


Fig. 4. Heart and ventilation rates as a result of stimulation by light and mechanical stimuli (i.e. water drop, pebble drop) (means  $\pm$  S.E.M.,  $n = 5$  individuals). Rates were measured before ('Pre') and after 1 min, 5 min and 10 min delay following the stimulus. Despite significant differences ( $*P < 0.05$ ,  $**P < 0.01$ ) in both HR and VR between animals there was a significant increase of HR after onset of light and of VR after mechanical stimulation ( $*P < 0.05$ ,  $**P < 0.01$ ; two-way ANOVA with repetitions).

height as for the water drop. This stimulus also induced a brief lengthening between beats for HR and VR followed by a rapid and robust increase in the HR and VR in four of the five animals within 2 min and in all five animals after 5 min (Fig. 3, right column, Fig. 4,  $P < 0.05$  Wilcoxon test). Some animals actually tailflipped backwards upon the pebble hitting the water. In such cases the large mechanical disturbances of the recording leads precluded the use of monitoring the HR and VR. In cases in which the animals would respond, without tailflipping, the animals would increase their HR and VR followed in most cases by some walking movement or a sweeping movement of their antennae.

### 3.2. Social interactions

Fig. 5 illustrates representative recordings of the HR and VR of two crayfish that were placed in the same observation aquarium over the course of an agonistic interaction. The two individuals were maintained in an aquarium with an opaque divider held tightly between the walls of the aquarium, at which time baseline HR and VR recordings were taken. After the divider was re-

moved the two crayfish would slowly explore the entire surroundings of the aquarium. When the two crayfish were in visual proximity one or both would perform a meral spread of their chelipeds. This visual stimulus is thought to provide a threatening posture to the opponent (Bruski and Dunham, 1987). In some cases, one crayfish would raise its chelipeds and the opponent would show no detectable difference in its behavior, but a dramatic increase in its HR and VR might occur. During intense battling with each other, both HR and VR in both individuals fluctuate quite drastically over short periods of time (Fig. 5). The changes in HR and VR are not always in synchrony with each other and may rapidly change independently. This is also illustrated in Fig. 2 since after the stimulus, when HR and VR slowed down, the HR picked back up quicker than the VR. In general there is a coordinated activity profile over several minutes between the HR and VR (Wilkins, 1976; McMahon, 1995) but not always within short intervals. The animals were deemed to be either dominant or subordinate by the end of the agonistic encounter (Fig. 6). The same criteria were used to determine the social status as in earlier studies (Bruski and Dunham,

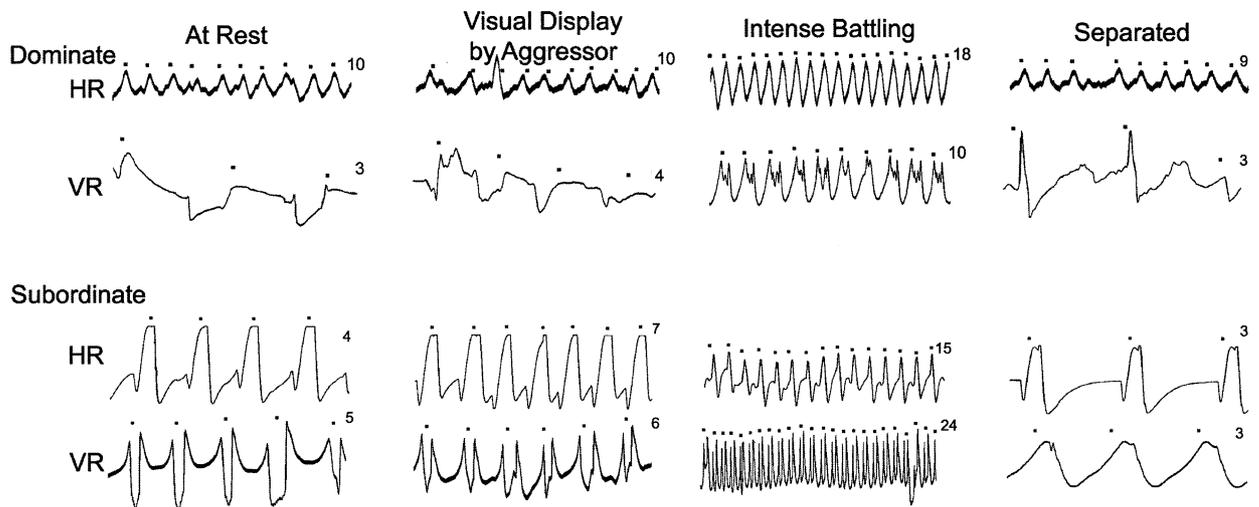


Fig. 5. Representative recording for 10 s of HR and VR during social agonistic interactions of crayfish. The responses observed reveal that the animals HR and VR are related to the type of interaction and the degree of physical effort during an agonistic interaction. The behavior of one crayfish displaying a meral spread of its chelipeds results in the opponent crayfish to increase its HR and VR, even though no obvious behavior change can be detected during this period for this crayfish. During a battle both crayfish may rapidly have change its HR or VR independent of the other. For example, the aggressive crayfish had a short burst of increased VR without as large of a change in its HR, whereas the submissive crayfish during the same period showed just the opposite change. It does not appear that a subordinate or dominate distinction can be made based on differences in HR or VR measures even when one animal is clearly dominate to the other. A dot is placed above each event along with the count for the 10-s recordings. These values if multiplied by 6 will give BPM for the window of time.

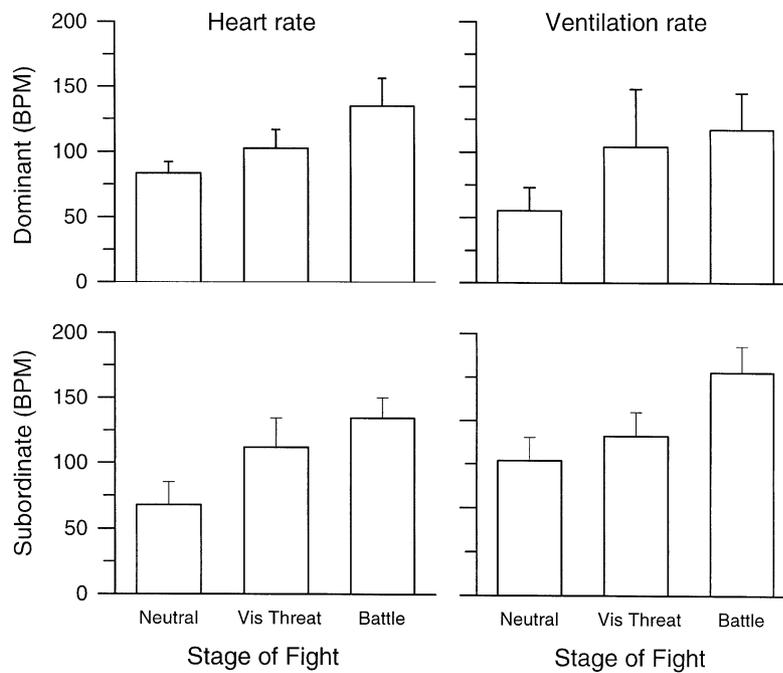


Fig. 6. Heart and ventilation rates of five pairs of crayfish at different escalatory stages of the fight (means  $\pm$  S.E.M.,  $n = 5$  individuals). Despite significant inter-individual differences both heart rate and ventilation rate were significantly increased during visual threats and battling as compared to neutral behavior (two-way ANOVA with repeated measures).

1987; Listerman et al., 2000). No consistent indication within the HR or VR recordings would indicate which animal would be the successor or the loser of an agonistic encounter. The general pattern from four dyad interactions is that alterations of HR and VR occur in the crayfish when they are in clear view of an opponent that raises its chelipeds as a visual display. When the two crayfish come in close contact and physically touch, even without extreme bodily movements such as walking or grabbing at each other, both individuals will have a higher HR and VR. From such interactions there is a larger fluctuation in VR than for HR. When the animals separate on their own or if they have been experimentally separated by a opaque divider in their aquaria, HR and VR can return to basal levels within minutes depending on the animals ambulatory behavior. The intensity of the battles is revealed in the physiological recordings of HR and VR, but since the battles vary in their duration and heightened intensities, it has proven difficult to quantify correlations of the averaged physiological measures to the particular components within the agonistic behavioral acts. Taking the average response during a behavioral act we have noted

that despite significant inter-individual differences both heart rate and ventilation rate are significantly increased during visual threats and battling as compared to a neutral behavior when the animals were not physically or visually interacting (Fig. 6, two-way ANOVA with repeated measures).

In one situation, in which HR was monitored but not the VR, two individuals were battling and one individual grabbed hard and twisted a cheliped of the opponent, resulting in the animal to autotomize the limb. During this battle the HR was highly elevated but as soon as the cheliped was autotomized HR stopped and remained flat-lined for approximately 30 s, after which time it slowly recovered in bursts of activity followed by pauses of a few seconds until it fully recovered (Fig. 7).

#### 4. Discussion

The results demonstrate that a rapid alteration in HR and VR can be induced by changes in the animals environment. In standardized experimental conditions of delivering stimuli to the crayfish's

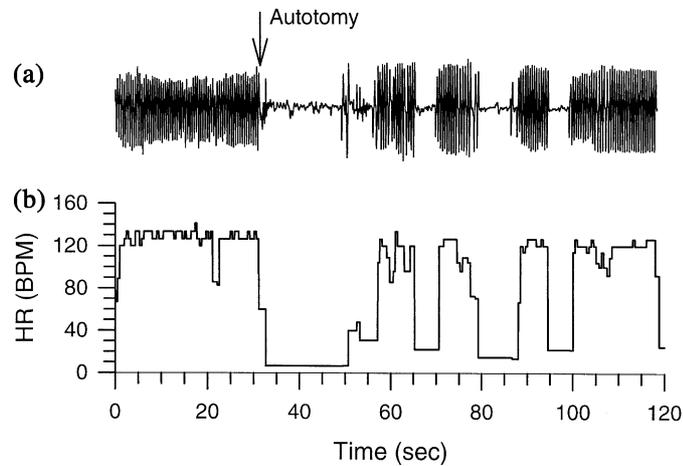


Fig. 7. HR measures prior, at the time the cheliped was autotomized, and afterwards in a agonistic battle. The recording reveals a rapid cardiac arrest upon limb loss followed by a bursting behavior of the HR (a). This reveals that HR can be induced to stop for approximately 30 s after limb loss, with shorter quite pauses between bursts of activity. The running average between beats (b) illustrates that the HR can rapidly return to high levels intermittently during the bursts.

environment, it became apparent that the physiological measures of HR and VR are more sensitive indicators than behavioral observations to identify if an animal is receptive to a stimulus. Three types of stimuli (the switch from dark to light, a drop of water, a dropped pebble) were used to induce a physiological or behavioral response to demonstrate that HR and VR serve as a reliable bioindex of the internal status which behavioral observation fail to provide. In addition, the alteration in VR and HR may be indicative of an animal's intention for body movement before physical movement occurs. As with the induced disturbances in the crayfish's environment, it is sometimes not apparent with behavioral observations that an animal is responding to the presence of another crayfish even when one of the individuals may be displaying a threatening posture. However, it is now apparent that HR and VR are sensitive indicators of the animals perception of any changes in its immediate surroundings. In addition, the measures demonstrate the animal's state of arousal during an agonistic social encounter. These physiological measures can also provide information on the duration of an altered internal state from such encounters. In the case of severe bodily injury, such as when the crayfish autotomized its claw during a battle with another crayfish, the reduction of HR could be a physiological adaptive response to avoid excessive blood loss.

Ethologist should note that from behavioral

observations one could draw the conclusion that the animals do not respond to these stimuli since no apparent behavioral changes are observed, but indeed the animals are very aware of the alterations in their environment and bodily injuries. HR and VR recordings portray a complex physiological awareness. Possibly it is not beneficial for an animal to relay, by behavioral means, how agitated it may really be under certain circumstances but instead to be ready to deal physiologically with whatever quick demands are put on itself for fighting or fleeing. On the other hand, there are times when one individual may wish to inform others of its social status or individual identity. Lobsters, for example, release urine during fights coupled with offensive behaviors providing the opponent with olfactory information about its aggressive state and identity (Breithaupt et al., 1999; Breithaupt and Atema, 1993, 2000).

The responses of the cardiovascular and respiratory systems in crayfish to environmental and socially imposed alerting stimuli are very similar as the responses of vertebrates mediated by the autonomic nervous system (Astley et al., 1991). For example, an acoustic induced startle response in rats produces a decrease in HR before an increase (Richardson et al., 1996). Also, the anticipation of an aggressive posturing in baboons results in an increase in HR (Smith et al., 2000). It is likely the selective pressures which promoted the development and maintenance of autonomic responses in the crayfish are the same for higher

animals. How the body plan developed such a system of course may have been different, but one would expect some similarities since the responses are neurally driven and regulated. In addition, blood borne hormones or compounds are released that influence many target tissues at once. One would also expect a similarity among animals in the types of target tissues to be affected and their responses which would allow an animal to best prepare the whole body for any potentially immediate stress. The cardiovascular and respiratory systems are two that are applicable to invertebrates as well as vertebrates.

Survival of an animal in an ecological arena requires the use of many predation-avoiding or combative behaviors. Field and laboratory observations indicate that crayfish exhibit a wide range of rapid behaviors to combat threatening stimuli as well as changes in the environment, thus indicating an ability to assess and respond to the circumstance. The ability to prime one's circulatory system with oxygen and to enhance blood flow at the same time demonstrates the coordination of these two complex tasks even before a real physiological demand is imposed, which is fitting since respiratory function is essential. As Wilkens (1976) stated, 'Respiratory exchange in decapod Crustacea requires the coordinated activity of the heart and the scaphognathites, appendages which ventilate the gills'. This alone implies that there is a strong potential survival advantage for such an ability to exist. Such responses may have indeed influenced further evolutionary strategies to arise in predator-prey behaviors. Since various degrees of a response can be initiated by an individual under differing physiological and environmental conditions, it leaves one to speculate that motivation of the animal may have a role in how an animal responds. Early behavioral studies in crustaceans have noted the existence of rapid defense reactions in crayfish (Bethe, 1897; Huxley, 1880; Wiersma, 1961). In this report and that of others it is convincing that crayfish demonstrate the 'fight or flight' type of response during social interactions, as well as to other stimuli in the environment, which gives support to the idea of an autonomic-like reflexive control in crustaceans.

There has been an interest in the past to anatomical comparisons of a putative autonomic nervous system (ANS) in arthropods to that of higher animals. Such work was primary to that of the Russian comparative neuroanatomists, Zavarzin

(1941) — his book was published in this year but the studies were conducted around 1910) and his collaborators (Orlov, 1927, 1929; Mewmyvaka, 1928 — see Zavarzin, 1941 for references). He drew direct analogous anatomical features of insect nervous systems to that of vertebrates. In his view, there are similarities between the sympathetic nervous system of vertebrates and the unpaired nerves of insects. He postulated that the ANS in the crayfish is located in the anterior and caudal parts of the animal's nervous system.

There is a similarity in the neural control of cardiac and ventilatory systems between decapod crustaceans and vertebrates. The cardiac ganglion is in close physical association with the heart and consists of a small number of neurons (Alexandrowicz, 1932; Maynard, 1961; Cooke, 1988; Kuramoto and Yamagishi, 1990). The ganglion is under the continuous control by inhibitory and acceleratory neurons located in the subesophageal ganglion. The regulation of ventilatory rhythm is also located in the subesophageal ganglion (the situation similar to that in the medulla oblongata of vertebrates). Moreover, the activity of circumesophageal connectives (which are associated with input and output of the subesophageal ganglion) can be altered by sensory inputs which parallel changes in HR and VR (Wilkens et al., 1974; Field and Larimer, 1975a,b; Taylor, 1982). Comparative studies in other crustaceans also demonstrate tight neural regulation of cardiac performance (Miyazaki et al., 1985; Tanaka and Kuwasawa, 1991; Okada and Kuwasawa, 1995).

In addition to the direct neural control of the cardiac and ventilatory generators, crustaceans possess a highly elaborated system of the so-called neurohemal organs (specialized structures in which nerve terminals come into contact with the circulatory system) which release a number of substances. These structures may be analogous to the adrenal glands of vertebrates, since these two are designed for rapid release of substances, which leads to alterations in sensory and motor function, thus altering the behavior of the animal (Strawn et al., 2000). Rapid whole organism effects can be initiated in a humoral manner while more direct effects can be insured with direct innervation. The integration of the various inputs on a target tissue can be complex when considering modulation of both pre- and post-synaptic sites (Southard et al., 2000). It is well documented that

5-HT and OA have rapid effects on target organs which provide the functions required for behavioral responses and that they are released from the neurohemal organs. 5-HT is directly excitatory to HR, sensory neurons, most all motor neurons, and for all synapses investigated. It can enhance the vesicular release process involved with chemical synaptic transmission, whereas OA has been shown to have varied responses (Djokaj et al., 2001). Interestingly it has recently been shown that 5-HT can induce the release of the crustacean hyperglycemic hormone (CHH) from the eye stalk (Lee et al., 2000). This would fit well into the scheme of a stress like response, since release would promote a rapid increase in the circulating levels of glucose for use by all tissues.

Such differences in an animals state of being, assayed by HR and VR measures, may be useful to be able to predict behavioral patterns. It is known that rapid alterations in an animals behavior can occur due to the presence of a predator or bodily injury that definitely mark the animal, in such a manner, that it no longer responds in the same way to a given stimulus (Lang et al., 1977; Kellie et al., 2001). Such changes may come about because of an altered autonomic function, which then could have a long lasting consequence on the animal's behavior. Future investigations in the mechanisms of altered autonomic neuronal function in relation to previous history would be useful.

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