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Metabolic consequences of living in a wave-swept environment: Effects of simulated wave forces on oxygen consumption, heart rate, and activity of the shell adductor muscle of the abalone *Haliotis iris*

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Abstract

Animals in wave-exposed habitats must constantly contend with the hydrodynamic forces of lift and drag. In this study, we investigated aspects of the metabolic response of *Haliotis iris* to simulated wave forces varying in magnitude up to 9.6 N applied to the shell at 69° to horizontal, alternately from anterior and posterior directions, with a period of 10s. Shell adductor muscle activity (electromyogram, EMG), heart rate, and oxygen consumption were monitored during force application and during extended recovery. EMG spiking was absent at zero force, but increased markedly with increasing force, in synchrony with the wave cycle. In contrast, heart rate was unaffected by wave forces and varied by only 5% over the whole range of applied forces. During force application, oxygen consumption increased by 10–25% above resting rates and remained elevated throughout a 5-hour recovery period, indicating a switch to anaerobic metabolism. It is concluded that living in a wave-swept environment is metabolically costly for abalone although this may be compensated by improved food availability and more efficient ventilation induced by external flow.

Keywords: Abalone; *Haliotis iris*; Metabolism; Wave force

1. Introduction

The rocky intertidal zone is a high-energy environment that can support high levels of productivity (Leigh et al., 1987) and species diversity (Dayton, 1971; Connell, 1972; Levin and Paine, 1974; Paine and Levin, 1981). It is also a very complex environment with several factors that affect organism size and abundance. On one hand, organism size can be enhanced by wave action. This is often the case for algae and sessile invertebrates for which wave action can increase food and nutrient availability, enhance gas exchange, and reduce predation and competition (Leigh et al., 1987). Conversely, the size of sessile organisms may be restricted in very exposed habitats due to mechanical limits imposed by hydrodynamic forces (Denny et al., 1985). Motile animals are similarly affected in the wave-swept intertidal and face additional challenges due to the effects of hydrodynamic forces on locomotion. Many animals are less tenacious while moving and face a greater chance of being dislodged while foraging (Menge, 1974; Menge, 1978; Denny et al., 1985; Denny and Blanchette, 2000).

Abalone (family Haliotidae) exist worldwide and are found in a range of habitats from temperate rocky coasts to tropical reefs (Geiger, 1998; Lindberg, 1992). There are approximately 56 species of abalone (Geiger, 1998) that vary widely in maximum length. The largest species, which include *Haliotis iris* (Martyn), occur in high-energy temperate shorelines where there is an abundance of drift macroalgae (Estes et al., 2005). In this environment, abalone employ a "sit-and-wait" foraging strategy in which they remain relatively sessile and trap algae that drift by under the large foot. *H. iris* inhabits rocky coasts of New Zealand.

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The adults are typically found on flat boulder bottoms, low rocky shelves, and the bottom of vertical walls (Poore, 1972a) and thus are exposed to the constant hydrodynamic forces that bring drift algae to it.

Although abalone tend to grow larger in more exposed habitats, they may be the exception rather than the norm. Marine snails inhabiting exposed sites generally tend to be smaller than those found in protected areas, with thinner shells and larger feet (Boulding, 1990). These adaptations presumably help them avoid dislodgement. A number of studies have documented size differences and growth rates between conspecific gastropods in different habitats (Brown and Quinn, 1988; Etter, 1988, 1989; Boulding and Van Alstyne, 1993; Hobday, 1995) and have also shown that snails transplanted from exposed sites to more protected habitats exhibit increased growth (Brown and Quinn, 1988; Etter, 1988, 1989; Boulding and Van Alstyne, 1993).

Although differences in size have been well documented in gastropods, the physiological or mechanical bases for these differences are unresolved and probably multiple. Gastropods adhere less tenaciously to the substratum during locomotion (Miller, 1974) and wave forces can limit the ability of mobile animals to forage (Menge, 1974; Menge, 1978). Thus, some authors suggest that reduced caloric intake limits growth in gastropods that actively forage (Brown and Quinn, 1988). Denny et al. (1985) attribute size variations of organisms in wave-swept areas to increased mortality rates of larger individuals due to increased mechanical forces of drag and lift causing higher levels of dislodgement (but see Denny, 1999). However, differences in size of wave-exposed gastropods could also be due to the increased metabolic demands of resisting dislodgement by hydrodynamic forces. Even for abalone, with their efficient "sit-and-wait" foraging, living in a wave-swept environment must have metabolic costs.

Gastropods adhere to the substratum by secreting from the sole of their muscular foot a thin layer of adhesive mucus which acts as a glue when the animal is stationary (Denny and Gosline, 1980; Denny, 1984). In limpets and abalone, the tarsos muscle of the foot is closely associated with an adductor or columellar muscle which dorsally attaches to the shell and is responsible for clamping the shell tightly against the substratum (Trueman and Brown, 1985; Voltzow, 1990). In limpets, adhesion is accomplished by an interaction between the mucus layer and contraction of the columellar muscle resulting in both suction and mucus-derived adhesion (Smith, 1991, 1992). The extent to which muscular contraction contributes to adhesion and the energy costs associated with these different mechanisms are unknown.

The purpose of this study was to investigate the effects of simulated hydrodynamic forces on the metabolism of gastropods. The abalone, *H. iris*, was chosen for its preferred habitat and large size. It is a large gastropod, reaching 180 mm length (Poore, 1972c; Geiger, 1998), which enabled us to monitor multiple aspects of its metabolic response. Specifically, we measured heart rate and adductor muscle activity while a range of forces were applied at constant period, mimicking waves of various magnitudes. We also measured oxygen consumption during application of forces and during an extended recovery period to investigate the prolonged effects of wave exposure.

2. Materials and methods

2.1. Animal collection and maintenance

Abalone were collected from South Bay, Kaikoura, New Zealand and transported in moist air to the University of Canterbury, Christchurch, where they were held in a recirculating seawater system at 15 °C and a salinity of 34–35‰. All experiments were conducted within 6 weeks of collection. The abalone were fed a mixture of artificial abalone food and powdered seaweed.

2.2. Morphometric measurements

To determine average areas required for estimating lift and drag on the abalone, morphometric measurements were made on a representative size range of *H. iris* used in our study (N=15; 102–141 mm length). Shell height, width, and length were recorded directly using Vernier calipers; measurements of width and length were taken at the largest appropriate diameter of the inferior shell margin. Measurements of shell areas were made by digitally photographing each animal from the front, back, and ventral aspect (taken through a glass aquarium onto which they were allowed to adhere). The calibrated photographs were traced using ImageJ (National Institutes of Health, Bethesda, Maryland, USA). The measurements of area included shell aperture area (A_a) and shell frontal area (A_f, the projected area of the face of the shell in the plane perpendicular to the direction of flow). All areas from photographs were traced twice, recalculated, and averaged.

2.3. Experimental apparatus and determination of experimental forces

To test the effects of wave force action on abalone metabolism, a machine was constructed to mimic oscillatory wave forces in a laboratory setting (Fig. 1). Period, force, and the angle at which the force was exerted could all be manipulated. Briefly, individual abalone were held in a temperature-controlled seawater bath under the machine. Two lengths of wire with springs (A) attached to them were connected, at one end, to a hook attached mid-dorsally to the shell and, at the other end, to a peg (C) on each of two motors (B) clamped to a metal rod arching over the seawater bath. The pegs traveled in separate circles producing a sinusoidally varying tension on the springs.

"Wave" period was manipulated by controlling the time for each motor to complete one revolution. In the present study, each motor alternately completed its revolution in 5 s, simulating a wave washing over the animal and back again with a period of 10 s, which is a typical period for the east coast of New Zealand (Pickrill and Mitchell, 1979). "Wave" force was manipulated by using springs of different stiffness and by adjusting the distance of the attachment peg from the motor axis. The diameter of the circle traveled by the peg on the motor was kept as small as possible to minimize deviations from the desired angle as the force was exerted. Angle deviation was

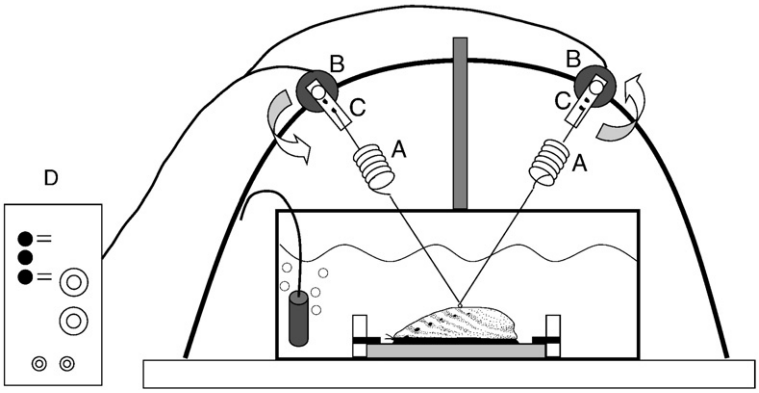

Fig. 1. Diagram of the machine used to vary magnitude of force exerted on the abalone. Wires and springs (A) were attached to pegs on bars (C) connected to two motors (B) which caused the bars to rotate and produce sinusoidally varying tension on the springs. On the other end, the wires were attached to the shell of the abalone via a small stainless steel hook. When each bar turned, the spring was stretched a distance equal to the diameter of the circle outlined by the peg. Thus the magnitude of force could be adjusted by using various springs and controlling the diameter of the peg rotation. The controller box (D) was used to set the rotation time of the motors such that the period of rotation of each motor was 5 s, yielding a 10 s period for the two combined. See text for more detail.

approximately 2°. The angle of the applied force was manipulated by sliding the motors along the metal arc.

Lift and drag forces at water velocities that *H. iris* might encounter in its wave-swept habitat were determined from the morphometric measurements. Acceleration reaction is small compared to drag for small organisms at the velocities used (Denny, 1989; Gaylord, 2000) and was ignored. The force of drag, F_d, was calculated from:

$$F_d = 0.5\rho v^2 A_f C_d \quad (1)$$

where C_d is the coefficient of drag, ρ is the density of the fluid (in this case sea water, $\rho = 1025$ kg m^{-3}), υ is the velocity of the fluid, and A_f is the shell frontal area. The force of lift, F_t, was calculated from:

$$F_l = 0.5\rho v^2 A_a C_l \quad (2)$$

where C_l is the coefficient of lift and A_a is the aperture area of the shell projected in the direction of lift. C_d and C_l are dimensionless values which are functions of the shape of the object in question and the Reynolds number of the system. They were treated as constants since their magnitudes change little over the Reynolds numbers used in this experiment (Denny et al., 1985). A value of 0.2946 was used for C_d, representing the average of values for *Haliotis rufescens* reported by Denny (1995). As there was no C_l reported for *H. rufescens*, a value of 0.2724 was used which represents a value similar to those of limpets with similar length: width ratios as *H. iris* (Denny, 1995). Lift and drag were calculated for a range of velocities and added as vectors yielding a single resultant force:

$$F_R = \sqrt{\left[(F_d)^2 + (F_l)^2\right]} \quad (3)$$

acting at angle θ from the substratum determined by the equation:

$$\theta = \tan^{-1}(F_l / F_d). \quad (4)$$

Thus, for *H. iris* experimental peak forces of 1.6 N, 3.2 N, 6.4 N, 8.0 N, and 9.6 N (corresponding to peak flow velocities of approximately 1.1, 1.6, 2.2, 2.5, and 2.7 m s^{-1}) were applied at an angle of 69° from horizontal.

2.4. Muscle activity and heart rate

2.4.1. Animal preparation

To determine the effects of different forces on adductor muscle activity and heart rate, electrodes were implanted through the shells of the abalone. Adductor muscle activity was monitored by electromyography (EMG) using copper electrodes inserted directly into the adductor muscle. Two small holes were drilled through the shell using a flexible-shaft power drill (Dremel, Racine, WI, USA) and a 1.5 mm drill bit. One hole was placed directly in the center of the abalone's shell which corresponds to the middle of the adductor muscle. The second hole was placed anterior to the first hole and slightly off center, about 1 cm from the first hole. Care was taken not to drill into the adductor muscle itself. This placement of the electrodes ensured that activity of dorsoventral fibers (those responsible for holding the shell next to the substratum) would be monitored since abalone adductor muscle is mostly composed of these fibers and they are abundant in the center of the muscle (Voltzow, 1990). Two 1 m lengths of copper wire (diameter 0.14 mm) were stripped of insulation on the last 10 mm of one end and the uninsulated portions were inserted through the holes and into the adductor muscle. The wires protruding from the holes were held in place with rubber strips secured with cyanoacrylate gel adhesive and connected to a biophysical amplifier (Universal amplifier model 13-4615-58, Gould Inc., Cleveland, Ohio, USA). The EMG signal was recorded using a data acquisition system (PowerLab, ADInstruments, Bella Vista, NSW, Australia) and an associated data analysis program (Chart 5.0, ADInstruments).

To measure heart rate, two holes (1.5 mm diameter) were drilled through the shell on either side of the heart using a dental burr attached to the flexible-shaft drill taking care to avoid damage to the underlying epidermis. The insulation was removed from the last 5 mm of two lengths of copper wire

(0.2 mm diameter) and the uninsulated ends were coiled to prevent irritating the animal, inserted into the holes and secured with rubber strips and cyanoacrylate gel adhesive. Heart rate was monitored continuously using an impedance converter (UFI Model 2991, Morro Bay, CA, USA) connected to the data acquisition system.

After electrode implantation, the abalone were allowed to recover for at least 24 h before experimentation.

2.4.2. Experimental treatments

Abalone ($N=7$; 153–276 g) were placed onto rough ceramic tiles which were clamped under the wave simulator onto the base of a water-jacketed, Plexiglas testing chamber (15 cm × 27 cm × 15 cm) containing seawater maintained at 15 °C. Oxygen levels were maintained with an air-stone. Wires extending from the motors of the wave simulator were attached to a small, stainless steel hook previously screwed and glued (cyanoacrylate) into the shell of the abalone. The abalone remained in the chamber for a 2-hour settlement period prior to treatment. At the beginning of the treatment period, adductor EMG and heart rate were measured for 30 min to obtain baseline measurements. After this initial rest period, three force treatments (1.6 N, 3.2 N, and 6.4 N) were applied for 30 min each. The final force treatment was followed by a 30 min recovery period. Thus, each treatment lasted 2.5 h with three 30 min force periods bracketed by a baseline period at the

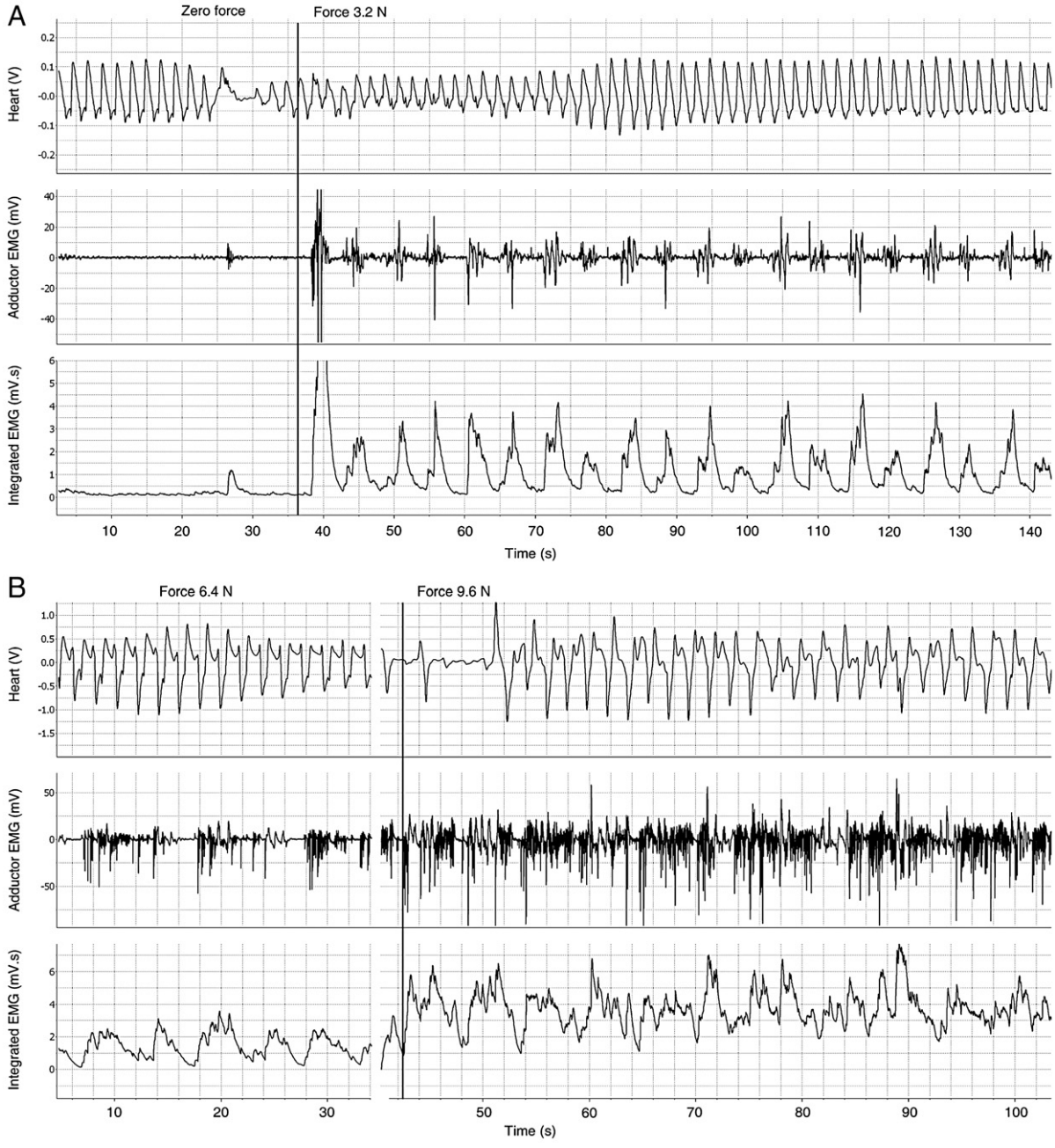

Fig. 2. (A) Record showing the effect of force application after an initial baseline period on heart rate, adductor muscle activity (EMG), and the integral of the EMG signal over time. (B) Record showing the difference between a lower force treatment and a higher force treatment.

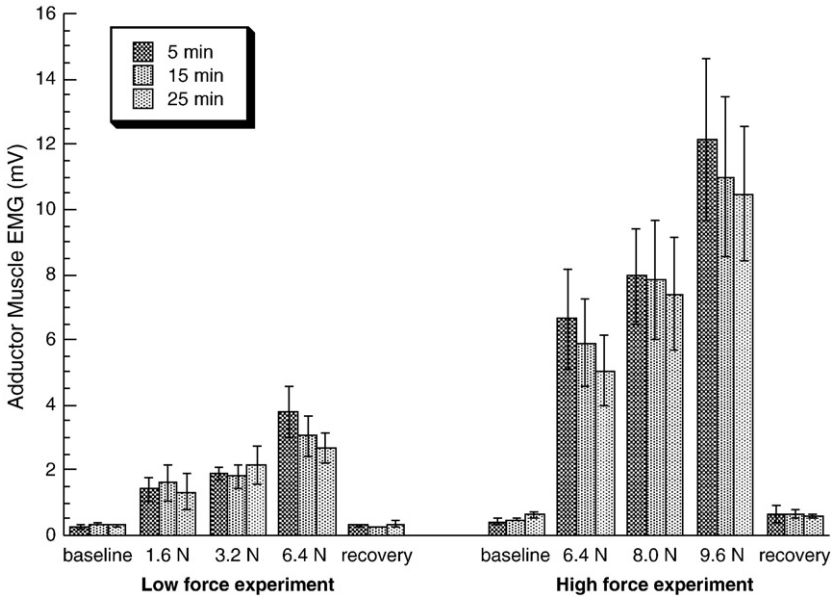

Fig. 3. Adductor muscle activity of *Haliotis iris* exposed to different magnitudes of force. The EMG trace was analyzed for three 2 min periods centered on 5 min, 15 min, and 25 min. Values are means±S.E.M.

beginning and recovery period at the end. The forces were applied in a balanced order such that each possible sequence of forces was used once and one was used a second time.

The experiment was repeated with a different group of abalone ($N=6$; 179–337 g) using forces of 6.4 N, 8.0 N, and 9.6 N, again with balanced treatments.

Mean muscle activity was determined by integrating the absolute value of the EMG signal over time (mV s). For illustration, this integral is shown (Fig. 2) with a 0.5 s time constant. Statistical analysis was performed on the mean slope of the integral (mV s s^{-1} = mV) for selected time periods. To determine if muscle activity changed during prolonged force application (e.g. as a result of sensory adaptation or habituation), the EMG trace was analyzed for three 2 min periods (centered on 5 min, 15 min, and 25 min) within each 30 min force application. Mean heart rate at each force was determined during the same 2 min periods and averaged for each 30 min force application.

2.5. Oxygen consumption

2.5.1. Animal preparation

To determine the impact of different forces on abalone oxygen consumption, each abalone was fitted with a mask from which exhalent water could be sampled and oxygen levels measured. Respiratory water flow over abalone gills is

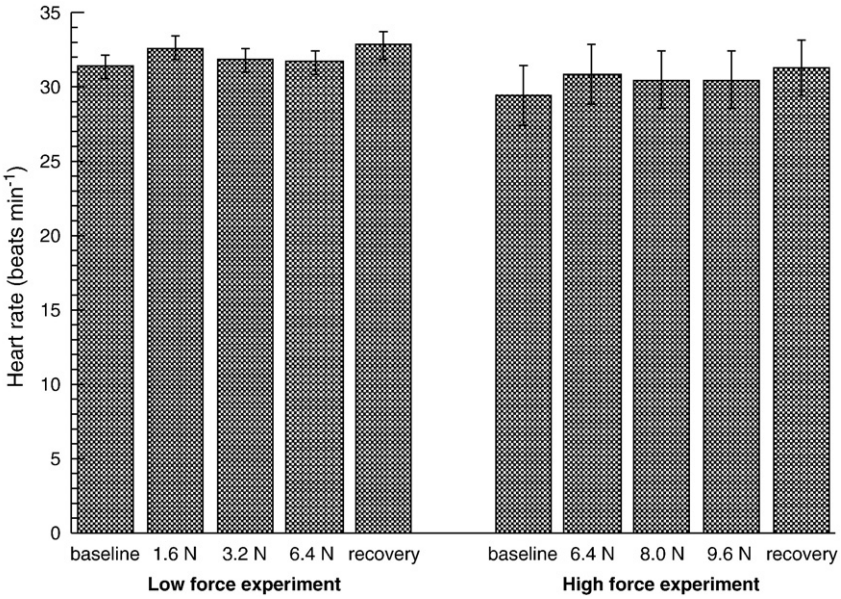

Fig. 4. Heart rate of *Haliotis iris* exposed to different magnitudes of force. Values are means±S.E.M.

unidirectional, entering over the abalone's head and exiting through holes (tremata) in the shell (Crofts, 1929; Yonge, 1947; Voltzow, 1983; Taylor and Ragg, 2005). The masks were made from pieces of PVC tubing (10 mm internal diameter) long enough to cover all of the tremata on the abalone's shell. The tubing was cut longitudinally to fit the contour of abalone's shell and the area where the mask was affixed was cleaned of epibionts and dried. The mask was first secured with cyanoacrylate gel and then sealed around the edges and at the front of the abalone with a hot glue gun. The posterior end of the mask was open and connected to a peristaltic pump. The pump produced a flow of 32 mL min^{-1} through the mask which was a ventilatory flow adequate for normal respiration of *H. iris* (Taylor and Ragg, 2005). Water was sampled (0.8 mL) from the mask into a glass syringe via a length of tygon tubing attached to a needle inserted into the mask. The oxygen tensions of the exhalent water and of the water bath were determined using an oxygen electrode (model 1302) in a 15 °C water-jacketed microcell (model MC100) and meter (model 781, Strathkelvin Instruments, Glasgow, Scotland).

Oxygen consumption (M_{O2}; μmol $O_2 g^{-1} h^{-1}$) was calculated from:

$$M_{O2} = (P_{bath} - P_{ex})V \cdot S/W$$

where $P_{bath} - P_{ex}$ are the P_{O2} (Torr) of the water bath and the exhalent water, V is the rate of water flow through the mask (L min^{-1}), S is the solubility of oxygen in seawater at 15 °C (1.62 μmol O_2 $L^{-1} Torr^{-1}$), and W is the wet mass (g) of the abalone including shell.

2.5.2. Experimental treatments

Abalone were divided into three treatment groups: those exposed to 6.4 N force (N=5; 166–274 g wet weight), those exposed to 9.6 N force (N=5; 150–290 g), and a control group that was not exposed to a force (N=5; 159–337 g). Individual abalone were placed into a small, open-top plastic container to limit mobility during the settling period. The container was clamped into the seawater testing chamber under the wave simulator and maintained at 15 °C. The mask was attached to the peristaltic pump and flow was maintained at 32 mL min^{-1}. Oxygen levels were maintained close to saturation using an air-stone. The wires extending from the motors of the wave simulator were attached to a shell hook as described above and the abalone allowed a 12 h settlement period prior to the treatment.

During each experimental trial, oxygen consumption was monitored over an 8-hour period. Resting oxygen consumption was determined during the first hour by sampling the exhalent water and the water bath in 15 min intervals. The wave simulator was then turned on and the abalone were exposed to either a 6.4 N force, a 9.6 N force, or no force (control) for 2 h. Water samples were taken every 15 min during this period. After the treatment, the abalone were monitored over a 5-hour recovery period in which water samples were taken every 15 min for the first 2 h then hourly for the remainder of the time.

To investigate whether the ventilatory flow rate produced by the peristaltic pump was limiting oxygen consumption, the flow rate was increased in a subset of animals from each treatment (N=2 from 6.4 N group, N=3 from 9.6 N group, and N=4 from control group) after the end of the recovery period. The flow rate was increased to approximately 57 mL min^{-1} and oxygen consumption was monitored every 15 min for an additional 2 h.

Values are reported ± the standard error of the mean unless otherwise noted.

3. Results

3.1. Muscle activity and heart rate

Magnitude of force significantly affected EMG activity in the adductor muscle. During the initial baseline period EMG

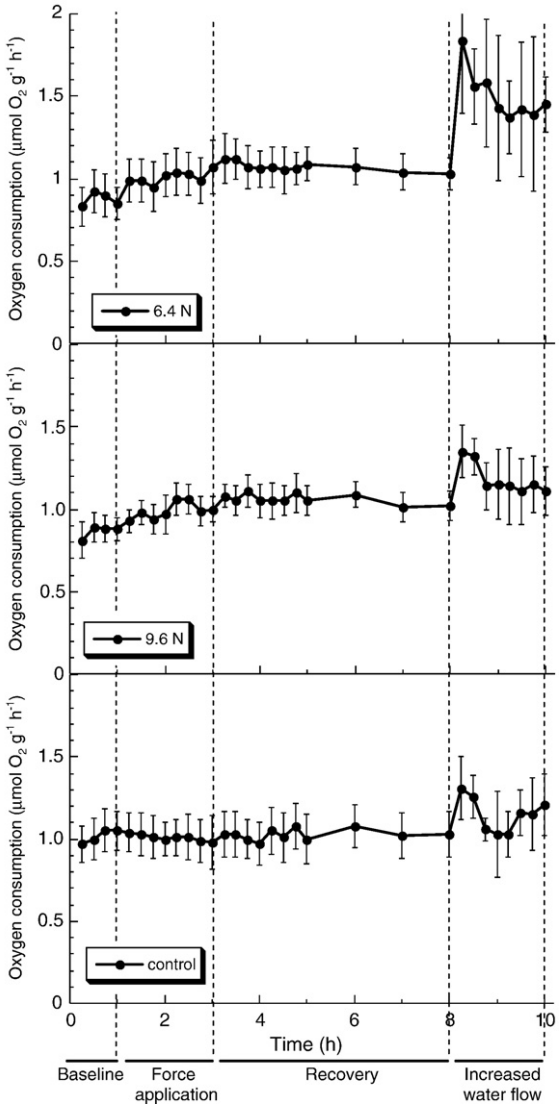

Fig. 5. Oxygen consumption of *Haliotis iris* exposed to 6.4 N force and 9.6 N forces, and of control animals not exposed to a force. Abalone were allowed to settle in the experimental chamber for 12 h prior to treatment. Force application occurred for 2 h after an initial 1 h period to establish baseline oxygen consumption levels. After force application, oxygen consumption was monitored for a 5-hour recovery period. At the end of the recovery period, water flow through the ventilatory mask on the abalone was increased to investigate ventilation limitation. Values are means ± S.E.M.

activity was essentially zero except for occasional small spikes associated with observed movements of the abalone as it shifted position in the tank. Brief larger spikes of activity were recorded if the abalone clamped its shell against the tile. However, with the onset of the wave simulation, EMG activity increased each time force was applied to the shell (Fig. 2A). At the lower force treatments, EMG activity returned to close to resting levels at zero force during reversals but at the higher forces, significant EMG activity was observed between half cycles (Fig. 2B).

In the low-force experiment, EMG activity significantly increased with an increase in peak force ($F_{4,24}=11.31$, $P<0.001$; ANOVAR; Fig. 3). Post hoc tests (Tukey's) indicated that there was overlap in the treatments. Essentially, the baseline, recovery and 1.6 N treatments formed one homogenous subgroup while the 1.6 N, 3.2 N, and 6.4 N treatments formed another subgroup. EMG activity also increased significantly with force in the high force experiment ($F_{4,20}=18.38$, $P<0.001$; ANOVAR; Fig. 3), although this time there was very little overlap between the treatments. Tukey's post-hoc tests indicated that the baseline and recovery groups formed one homogenous subgroup while the three force treatments were statistically different from them and from each other (all $P<0.05$).

EMG activity tended to decrease over the 30 min that a particular force was applied, especially at the higher forces (Fig. 3) but this trend was significant only for the 6.4 N treatment in the low-force experiment ($F_{2,12}=5.06$, $P=0.03$; ANOVAR).

In contrast, magnitude of force had little effect on heart rate. Although there were significant differences in heart rate at the low-force treatments ($F_{4,24}=6.90$, $P=0.001$; ANOVAR), heart rate only varied between 31.4±0.8 beats min^{-1} during the initial baseline period and 32.8±0.9 beats min^{-1} during the recovery period, with the force treatments falling in between these two values (Fig. 4). Heart rate did not change significantly in the second experiment using high force treatments ($F_{4,20}=2.54$, $P=0.072$; ANOVAR). In this experiment, mean heart rate varied between 28.9±1.9 beats min^{-1} and 30.6±1.8 beats min^{-1} (Fig. 4).

3.2. Oxygen consumption

In the controls and treatment groups, oxygen consumption increased slightly during the initial baseline period as measurements were commenced (Fig. 5). With the onset of force application, animals in the 6.4 N treatment exhibited increased oxygen consumption of 10–20%. Oxygen consumption continued to rise to about 25% above resting and remained elevated throughout the 5-hour recovery period. Analysis of the hourly means for this group indicated that these increases were all significantly different from the mean baseline oxygen consumption ($F_{7,28}=5.71$, $P<0.001$; ANOVAR). A similar trend was observed the abalone in the 9.6 N treatment. When the force application ceased, oxygen consumption increased by a further 13–20% and continued to rise during recovery. Again, the hourly means were significantly higher than the corresponding mean baseline oxygen consumption ($F_{7,28}=4.10$, $P=0.003$; ANOVAR). Oxygen consumption of the control animals remained constant at baseline levels and no significant rises were found ($F_{7,28}=0.91$, $P=0.5$; ANOVAR). During the recovery period, the percent increase over resting M_{O2} in both the 6.4 N and 9.6 N treatment animals was significantly greater than that of the control animals ($F_{2,12}=12.57$, $P=0.001$; ANOVAR; Fig. 6), although a Bonferroni post-hoc test indicated that the 6.4 N and 9.6 N treatment formed a homogenous subgroup.

Increasing the flow rate of water past the gills caused oxygen consumption to increase sharply then decrease in all treatments (Fig. 5). However, after 2 h of increased flow oxygen consumption remained elevated compared to levels after 5 h of recovery in the abalone that had been subjected to force.

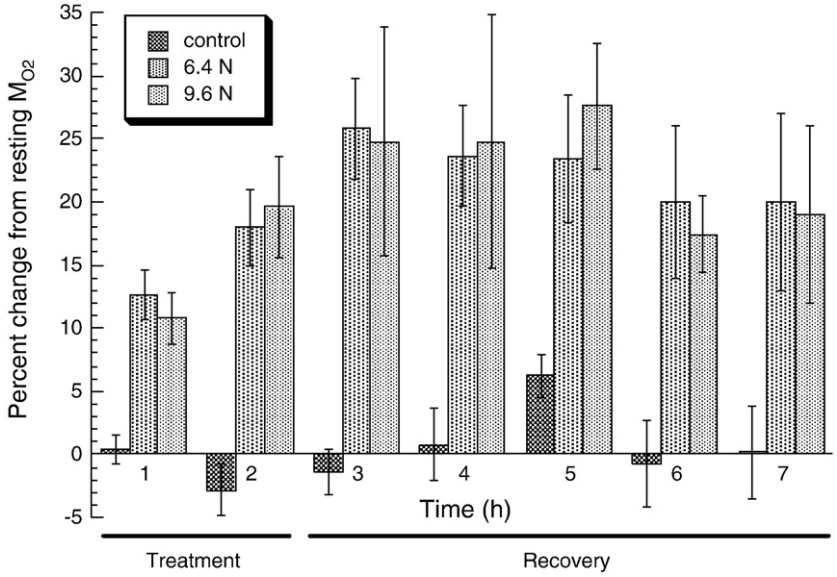

Fig. 6. Percent change from baseline oxygen consumption of *Haliotis iris* exposed to 6.4 N force and 9.6 N forces, and of control animals not exposed to a force. Values are means±S.E.M.

4. Discussion

Resisting dislodgement by oscillatory forces was a dynamic process for the abalone in this experiment, with periods of muscle contraction during force application and minimal EMG activity in the absence of force. The abalone displayed very little adductor muscle activity during the baseline period when no forces were being applied. With the onset of forces, muscle activity occurred immediately, synchronized with the applied forces (Fig. 2A). Furthermore, as the peak force increased so did adductor muscle activity (Fig. 3) and at the higher forces there was often substantial muscle activity at the zero point as the horizontal force component was reversed, indicating that the adductor muscle was continuously active (Fig. 2B). Attenuation of the EMG activity occurred during 30 min application of the highest forces (Fig. 3) consistent with habituation to repeated stimulation. However, the effect was minor and it appears that continual exposure to wave action would incur a substantial metabolic cost.

The adductor muscle is also involved in the "shadow reflex" where the shell is briefly pulled down towards the substratum when an abalone senses nearby movement. In *H. midae* (Trueman and Brown, 1985) and in *H. kamtschatkana* (Voltzow, 1986) this activity is associated with transient large pressure pulses (up to 3.4 kPa) within the muscle. The intermittent muscle activity recorded during initial baseline periods were apparently due to this behavior. These results indicate that abalone use adductor muscular contraction to maintain position on the substratum, but only as needed.

Changes in adductor muscle activity contrasted sharply with cardiac activity which maintained an essentially constant frequency of contraction throughout all force treatments (31.4 to 32.8 min^{-1} in the low-force experiment; 28.9 to 30.6 min^{-1} in the high force experiment; Fig. 4). Ragg and Taylor (2006) similarly found that *H. iris* heart rate remained constant at about 28 min^{-1} following a 1-hour period of environmental hypoxia and handling stress associated with emersion and cannulation for experimental purposes. However, they also found that blood flow increased by 2.5 times that of resting rates during this time and suggested that this was accomplished by increased stroke volume. Cardiac output was not measured in the present study but it is possible that changes in stroke volume compensated for increased activity induced by force application.

Measurements of oxygen consumption confirmed that resisting dislodgment increased metabolic energy expenditure of *H. iris*. The forces used in these experiments (up to 9.6 N applied at 69° from horizontal) mimicked relatively low wave velocities (up to 2.7 m s^{-1}) compared with maximal wave velocities that have been recorded (20 m s^{-1}; Denny et al., 1985; Denny, 1995) and velocities routinely experienced in the intertidal zone (5 m s^{-1}; Gaylord, 1999). Nevertheless, these relatively low forces increased oxygen consumption over resting values by 10–25% (Fig. 5, Fig. 6), and oxygen consumption remained elevated for the duration of the 5-hour recovery period.

The prolonged elevation of oxygen consumption during the 5 h recovery period following cessation of the force treatments suggests that the abalone were supplementing aerobic energy expenditure with energy derived from anaerobic sources and were repaying an oxygen debt. *H. iris*, like other abalone species, can support activity using energy produced by anaerobic glycolysis. For example, while righting repeatedly over an extended period of time, an action that requires regular contraction of foot and adductor muscles, *H. iris* exhibited significantly increased levels of D-lactate and tauropine, the principal anaerobic end products in abalone, in both foot and adductor muscles (Baldwin et al., 1992). During environmental hypoxia associated with prolonged emersion, these metabolites accumulated in these tissues both intracellularly and extracellularly (Behrens et al., 2002). Thus, it is likely that, in the present study, repeated contraction of the adductor muscle while resisting dislodgement also required input from anaerobic energy sources.

Correspondingly, Wells et al. (1998) found that *H. iris* living in an exposed habitat had increased levels of tauropine dehydrogenase (TDH) in both foot and adductor muscles compared to *H. iris* from a more sheltered habitat. TDH is the enzyme responsible for the production of tauropine during activity and increased levels in exposed populations imply that these animals regularly rely on anaerobic energy more than those from sheltered areas. The duration of increased oxygen consumption after cessation of force indicates that the abalone were still repaying an oxygen debt 5 h after treatment. This is consistent with the long recovery period observed in *H. iris* after hypoxia due to air exposure (Ryder et al., 1994), in which D-lactate levels required 48 h to return to resting levels, while tauropine levels did not return to normal until 120 h after treatment.

Unlike aerobic metabolism, anaerobiosis is not a steady state condition that can be maintained indefinitely. Thus it is unclear how repayment of the oxygen debt could be achieved under conditions of more or less continuous wave exposure. There is some evidence that *H. iris* migrates lower into the subtidal region during periods of very high wave activity such as during winter storms (Poore, 1972b). Although this might allow them to avoid the most intense wave action, the preferred habitat of *H. iris* is open coast where it is regularly exposed to substantial hydrodynamic forces.

It is important to note that although our apparatus simulated lift and drag on the shell due to wave action, the abalone experienced essentially static water conditions. Abalone are able to use induced flow from external sources to augment respiratory flow generated by gill cilia (Voltzow, 1983; Tissot, 1992; Taylor and Ragg, 2005) and there is evidence that in still water aerobic respiration of *H. iris* is ventilation-limited (Taylor and Ragg, 2005). Although, the artificial ventilation rate employed for respirometry (32 mL min^{-1}) exceeded the normal ciliary flow and was certainly adequate to support aerobic metabolism at rest, increased ventilatory flow may be required to support metabolism elevated by wave action.

A surge in oxygen uptake accompanied doubling of ventilation rate at the end of the 5 h recovery period (Fig. 5) and this elevation was sustained for at least a further 2 h after force treatments. This observation supports such a ventilation limitation, i.e. the initial peak represents reloading of oxygen-

depleted hemolymph followed by a period during which the oxygen debt was repaid at a higher rate. Ventilation limitation may also explain the similar rates of oxygen consumption recorded in abalone exposed to the 6.4 N and 9.6 N forces (Fig. 6) despite marked differences in adductor muscle activity (Fig. 3), i.e. the abalone relied on different levels of anaerobic metabolism to meet their metabolic needs. A more detailed study measuring both aerobic and anaerobic energy expenditure during force application would be needed to resolve this issue.

If the increased metabolism during force application found in our laboratory study is indicative of increased metabolic demands in wave-swept habitats, what are the ramifications for *H. iris*? It appears that even low wave forces can cause increased metabolic demands for abalone and it is likely that the much larger forces normally encountered would cause increased metabolism as well as increased chance of dislodgement. Both factors might be expected to limit growth rates and maximum size as predicted by Denny et al. (1985). Paradoxically, *H. iris* from sheltered habitats are generally smaller than abalone from more exposed locations (McShane et al., 1994) and exhibit slower growth rates (McShane and Naylor, 1995) possibly due to problems with siltation or inadequate food supplies. Like many abalone species, *H. iris* is able to trap drift algae under its large foot, and in wave-swept habitats this is their primary mode of feeding (Poore, 1972a). For *H. iris* it appears that increased hydrodynamic and metabolic demands incurred by living in exposed environments are balanced by increased food availability and enhanced ability to extract oxygen from the water.

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