

ASPECTS OF BRANCHIAL IRRIGATION IN THE LOBSTER *HOMARUS AMERICANUS*

I. FUNCTIONAL ANALYSIS OF SCAPHOGNATHITE BEAT, WATER PRESSURES AND CURRENTS

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INTRODUCTION

Normal branchial irrigation in decapod Crustacea is caused by the dorso-ventral pumping action of the scaphognathites. The scaphognathites are the enlarged blade-like exopodites of the second maxillae which move within the confines of the narrow pre-branchial chambers (Paterson, 1968). Water is pumped over the gills in a forward direction in most species including the lobster, but this is interrupted at irregular intervals by 'reversal beats' which propel water backward over the gills.

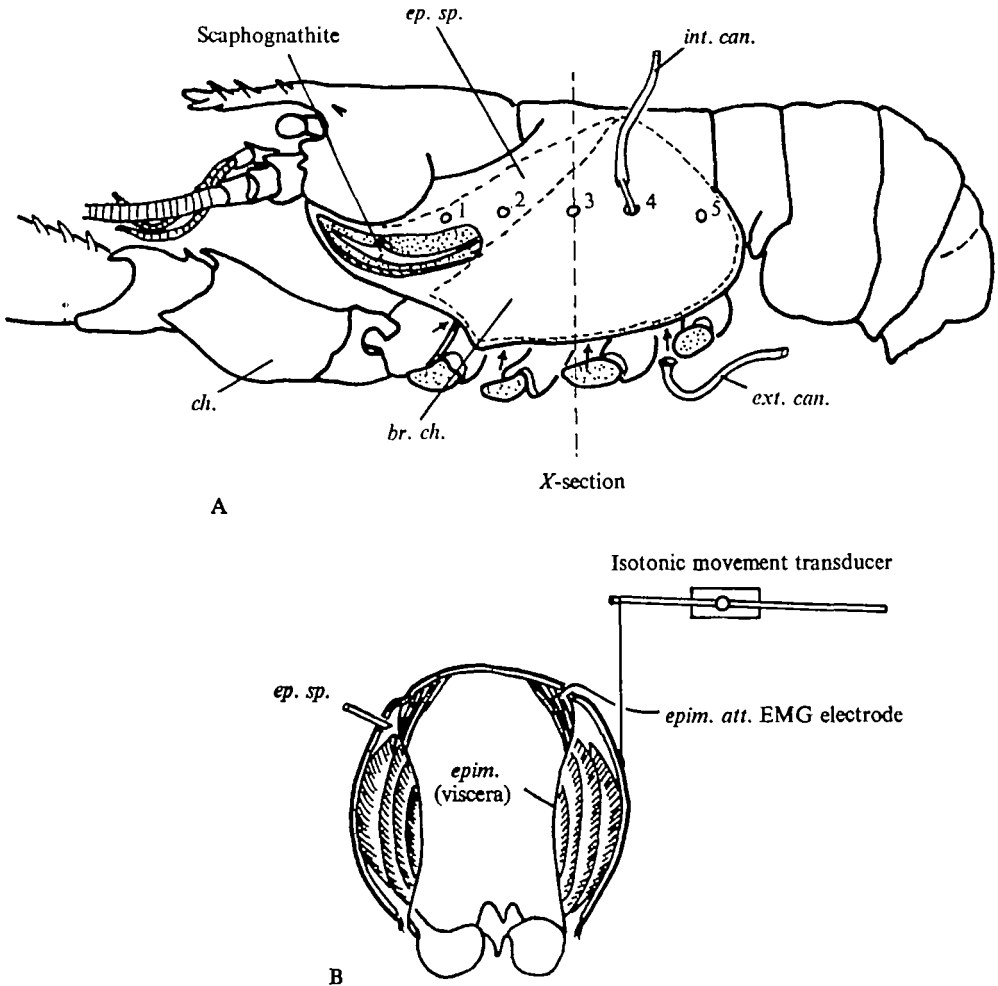
A number of studies (Arudpragasam & Naylor, 1964*a, b*, 1966; Hughes, Knights & Scammel, 1969; Larimer, 1961) have examined aspects of the hydrodynamics of branchial irrigation in crustaceans, but only Pasztor (1968) has examined the physiology of the pump itself. The latter study contained a brief description of the scaphognathite pumping mechanism and a detailed account of the scaphognathite neuromotor components. As yet there have been no analyses of the precise movements of the scaphognathite or of the hydrostatic pressures resulting therefrom. In this paper we present a photographic analysis of scaphognathite movements during forward and reversed beating plus simultaneous recordings of branchial pressures. The pressure profiles across the branchial chambers and the resultant irrigation currents through the gills are also described.

MATERIALS AND METHODS

Lobsters weighing 600–800 g were obtained commercially and maintained at 12 °C in running sea water. These animals fed vigorously and appeared normal in all respects. The chelae of all animals were removed at the autotomy plane several days before observations began in order to allow free access to the branchial chambers and scaphognathites. During experiments animals were clamped in a Plexiglas chamber similar to that described by Larimer (1961).

The movements of the scaphognathites were observed through celluloid windows which replaced the normal covering of the pre-branchial branchiostegite. The celluloid was heat-moulded to the shape of the carapace and glued in place with Eastman 910 adhesive by the technique of Hughes *et al.* (1969).

Hydrostatic pressures from positions inside the branchial chambers were recorded via PE 160 polyethylene cannulae connected to hypodermic needles inserted through holes drilled in the branchiostegites. External hydrostatic pressures were recorded by positioning cannulae under the ventral openings to the branchial chambers between



Text-fig. 1. (A) Diagram of lobster indicating the positions from which internal (numbers) and external (arrows) branchial pressure recordings were made. Also shown is the scaphognathite as seen through a celluloid window replacing the branchiostegite. (B) Cross-section through the thorax at position marked in (A) showing position of attachment of isotonic or isometric levers, pressure cannulae and EMG electrodes. *br. ch.*, Branchial chamber; *ch.*, cheliped; *epim.*, epimera; *epim. att.*, epimeral attractor muscle; *ep. sp.*, epibranchial space; *ext. can.*, external cannula; *int. can.*, internal cannula.

the thoracic appendages (Text-fig. 1 A). Hewlett-Packard 267 BC differential pressure transducers, d.c. coupled, were used to record pressures.

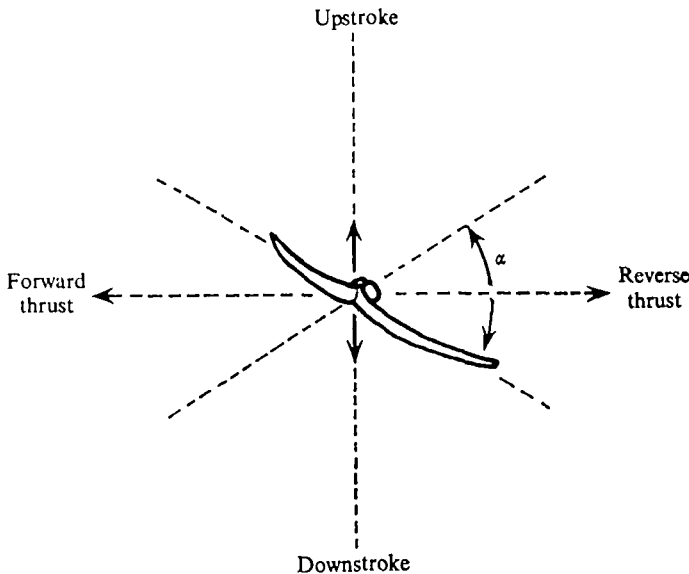
Movements of the carapace relative to the thoracic sternites which occurred during reversal beats were recorded by Harvard isotonic movement transducers or isometrically by an RCA 5374 transducer. The transducers were d.c. coupled and set so that a downward movement of the point of attachment resulted in a downward

deflexion on the recorder (Text-fig. 1B). Electromyograms (EMGs) were recorded via fine Teflon-coated silver wires (75 μm diam.) implanted into the body of a muscle, and were amplified by a Grass P-15 amplifier. Pressure, movement and EMG waveforms were recorded either on a Gilson oscillograph or were photographed from an oscilloscope.

Simultaneous photographic records of scaphognathite movements and the electronically recorded pressure and body movements were obtained by split-screen video cineradiography. The outputs from two video cameras with the opposite halves of the fields blanked out, one focused on the lobster and the other on the oscilloscope, were mixed through a Special Effects Generator giving a horizontally split composite image. This signal was first recorded on a video disk recorder, Data Memory Inc., Model 1008, which allowed 'freeze'-frame analysis of successive frames at $\frac{1}{30}$ th sec intervals. This information was next transferred to magnetic tape for storage. In the transfer from the disk to tape up to 10 sec of each successive frozen $\frac{1}{30}$ th sec frame was recorded. Playback through a TV monitor allowed single-frame and slow-motion visual analysis and still photography. Still photographs were taken of the TV display at $< \frac{1}{80}$ th sec shutter speed to avoid the vertical blanking bar artifact inherent in the video system.

RESULTS

In the lobster the scaphognathites normally act as suction pumps drawing water over the gills in a postero-anterior direction and expelling it from anterior excurrent canals located just below the antennae. As visualized in Text-fig. 2, the movements of



Text-fig. 2. Movements of the scaphognathite which occur only along the y -axis accompanied by changes in the attack angle (α). These movements propel water along the x -axis.

the scaphognathite can be resolved into movements in the y -axis during upstroke and downstroke accompanied by changes in the acute angle (α) between the x -axis and a line drawn through the scaphognathite, the attack angle. The angle of attack is

taken as positive if during upstroke or downstroke water was propelled forward, and negative if water was pumped backward. A complete beat cycle is taken to include both upstroke and downstroke. The number of complete beats per min. is the frequency. The scaphognathite movements produce a cyclic series of hydrostatic pressures resulting in the movement of water along the x -axis or thrust.

Forward beating

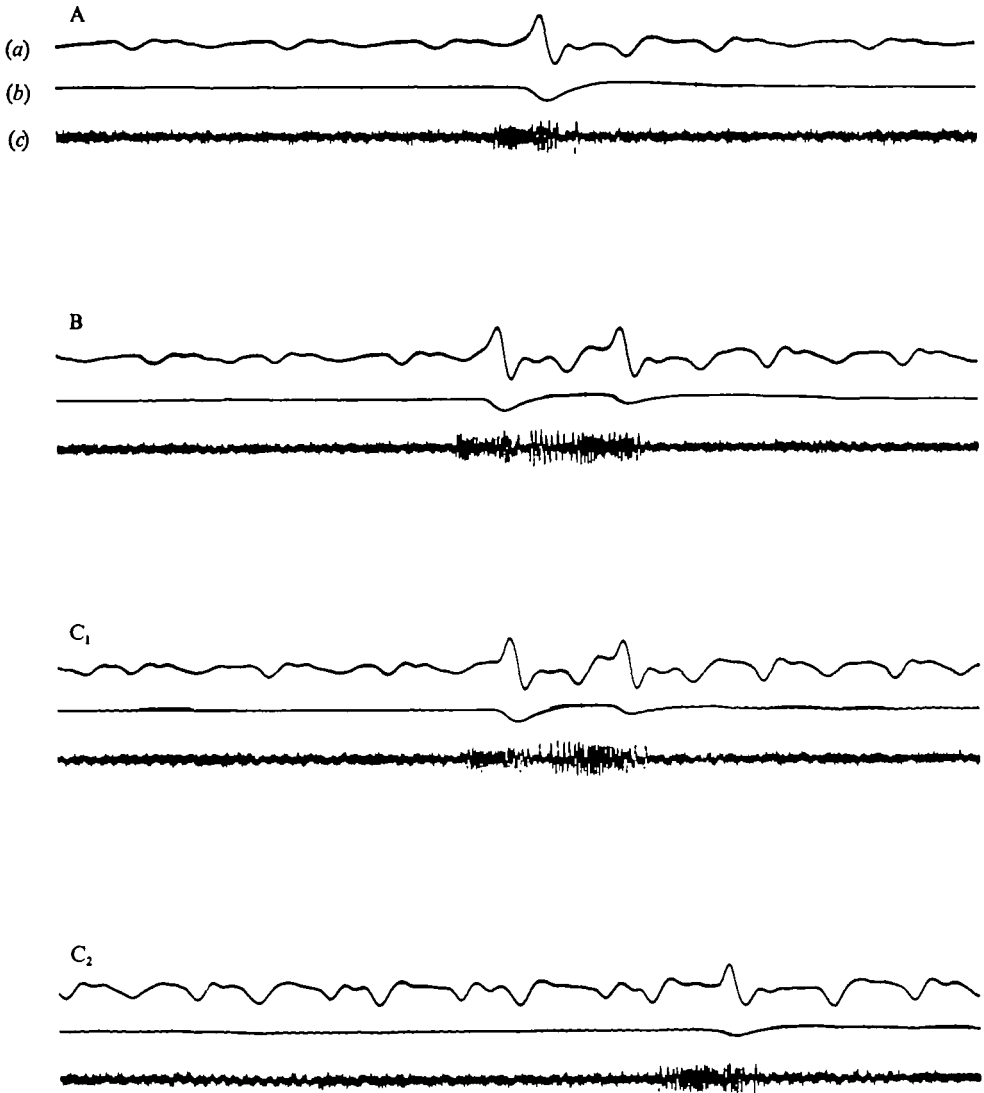
The movements of the scaphognathite, as viewed through a celluloid window, and the instantaneous hydrostatic pressures recorded from the gill chamber at position 2 (Text-fig. 1A) have been correlated by simultaneous recording on video tape. During forward beating (Pl. 1E-L) water is expelled during both upstroke and downstroke as the posterior portion of the scaphognathite first moves to close the pump chamber followed by the complete elevation or depression of the blade. Each half beat contributes to the maintained negativity in the branchial chambers. When the scaphognathites are at the upper or lower extremes of the half stroke the pre-branchial chambers are open, and as the blade pauses momentarily before moving in the opposite direction the water pressure moves upward toward a zero pressure difference between inside and outside the chamber.

Reversed beating

Normally single reversal beats occur randomly during the course of forward irrigation; they can occur unilaterally, but often simultaneous bilateral reversed beats are seen. The scaphognathite produces the positive pressure phase of the reversal waveform by assuming a negative attack angle during the upstroke of the beat (Fig. 3A-D). The examination of hundreds of reversals in several lobsters revealed that they occurred only during the upstroke of the beat and that even when they occurred in doubles or triples they arose from a fluttering type of beat in which there was an incomplete downstroke followed by a forceful negative attack-angle upstroke. During a reversal the force of the half beat was always great enough to drive the hydrostatic pressure of the branchial chamber positive and was of a force equal to or greater than the negative pressure of forward pumping.

Each reversal lasts only half a beat following which the branchial pressure is abruptly returned to the previous negative value (Pl. 1 and Text-fig. 3). There is no oscillation in the pressure following the quick return although some reversals are followed by a negative overshoot lasting for one or a few beats. A thoracic movement, consisting of a quick adduction of carapace to sternum, accompanies reversals. This movement occurs while the scaphognathite is stationary in the full up position (Pl. 1E). Rather than compressing the chamber and producing the positive pressure of a reversal this movement serves to re-establish branchial negativity. Text-fig. 3 shows that these movements arise at least in part from contractions of the epimeral attractor muscle whose primary function is to expand the branchial chamber on the side reversing by drawing the flexible dorsal aspect of the epimera medially. Contraction requires facilitation, yet shows the characteristics of a simple twitch (Text-fig. 3). The nerve command to the muscle consists of a burst of spikes which begins before the movement by 0.2-1.0 sec. The burst of potentials is followed by one or more spikes of large amplitude and longer duration. These late potentials may be inhibitory

thus allowing a quick relaxation in anticipation of another reversal. Indeed, when successive reversals occur during every scaphognathite cycle the amplitude of movement of the second contraction is less than the first indicating incomplete relaxation



Text-fig. 3. Branchial hydrostatic pressure (a), isotonically measured carapace movement (b), and EMGs from epimeral attractor muscle (c) recorded from same side during forward and reversal beats. Contraction of muscle coincides with the return to negative pressure in branchial chamber. A, B and C are from the same animal, C₁ and C₂ are continuous records.

between command bursts. The movement trace in Pl. 1 was recorded isometrically and the quick return seen during isotonic recordings was much delayed by the drag of the transducer. The movement of the whole carapace, as monitored here, merely reflects the movement of the epimera which runs from the carapace to the sternum.

Contraction of the epimeral attractor on one side caused a small movement on the opposite side, but had no effect on the opposite branchial pressure. In one lobster,

contractions of this muscle occurred in the absence of reversals and produced a brief increase in negativity with each contraction. In all other cases examined, however, the contraction of this muscle was coincident with the return to negativity following a reversal.

Several different stimuli were found to induce reversals. Brushing the hairs bordering the lower margins of the branchiostegites induced them more consistently than any other stimulation. This response was phasic and adapted quickly to continued stimulation of a given population of hairs. The response to first stimulation depended on the previous history of touching, being greatest after a rest. Reversals also occurred following the injection of milk near the incurrent openings and on two occasions occurred in long repetitive series immediately after a lobster was returned to water from air. The frequency of reversals showed no apparent correlation with oxygen tension or temperature (B. R. McMahon & J. L. Wilkens, unpublished observations).

Branchial pressure and water flow patterns

In order to gain some insight into the driving forces over the length of the gill chambers, internal hydrostatic pressure recordings were taken throughout the length of the branchial chambers and outside along the bases of the branchiostegites between the legs at positions indicated in Text-fig. 1 A. The internal pressure profile (Text-fig. 4 A) shows that during forward pumping the greatest negative pressures occurred just posterior to the scaphognathite and decreased in both directions from that point.

Table 1. *Relative volumes of water entering each of the branchial incurrent apertures*

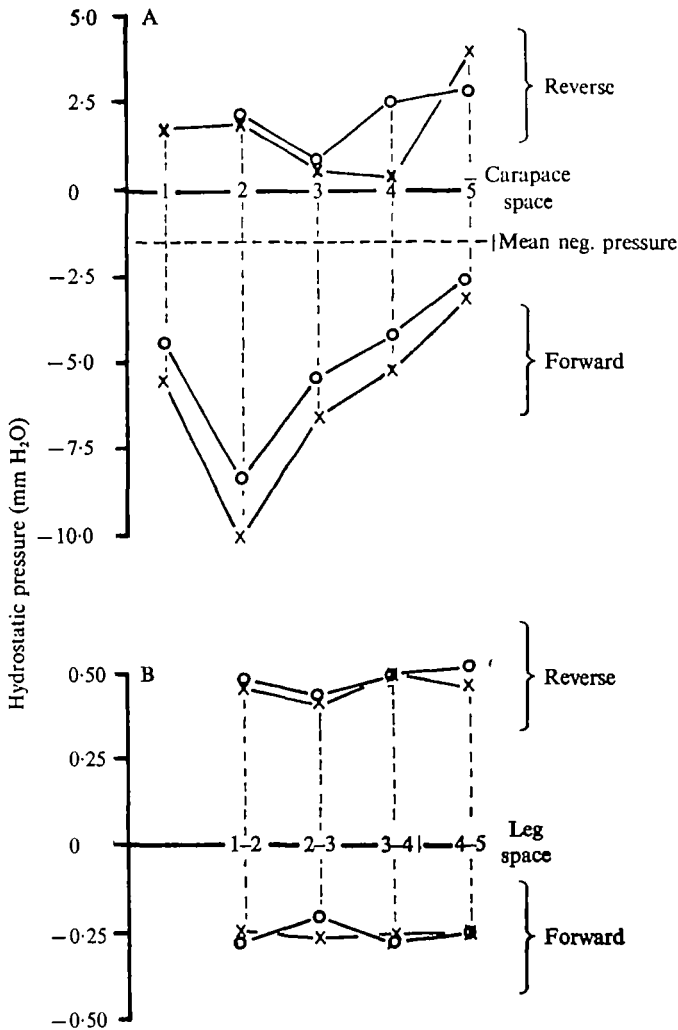
Position of cannula*	External hydrostatic pressure during forward pumping (mm H ₂ O)†	Area of incurrent aperture (mm ²)	Relative volume entering space/unit time (area × pressure)
Milne-Edwards space	—	Nil	Nil
1-2	-0.25	6.5	1.6
2-3	-0.25	8.3	2.2
3-4	-0.25	8.3	2.2
4-5	-0.25	7.5	1.9

* See Text-fig. 1 A.

† See Text-fig. 4 B.

During reversals the pressure profile became the steepest at the posterior border of the chamber. Text-fig. 4 B shows that the external pressures are rather uniform across the length of the chambers during both forward and reversed pumping. The much lower pressures recorded outside result from the difficulty of positioning the cannula precisely between the pereopods as well as the more diffuse nature of external water currents. The incurrent apertures between successive thoracic appendages are of similar areas (Table 1), while the Milne-Edwards spaces directly above the bases of the chelae are almost totally occluded in the lobster. If one assumes that the relative volume of water entering each incurrent aperture is proportional to the external pressure recorded in that space times the size of the aperture it is seen that approximately the same volume enters each aperture. This calculation is in good agreement with the conclusions drawn from observations of streams of milk or India ink through various parts of the branchial chambers as viewed through celluloid

windows. Water flow is also segmentally compartmentalized by the epipodite of each thoracic appendage. The epipodite is a thin-walled lamellate plate running dorsally between the gills with its lateral border closely apposed to the branchiostegite and its mesial margin closely apposed to the epimeral wall. Thus the water entering each incurrent aperture serves to irrigate only the gills of that thoracic segment.



Text-fig. 4. (A) Profile of hydrostatic pressures throughout length of branchial chamber. The records during forward beating represent maximum negativity and reversal pressure is peak positivity taken from the same recording. (B) Profile of hydrostatic pressures at ventral border just outside branchiostegite between appendages. Again forward and reversal pressures were taken from the same experiment and were recorded simultaneously with the internal pressures in (A). Positions of recordings as indicated in Text-fig. 1 A. Temperature, 12-13 °C. x and o are different lobsters.

The internal pressure is viewed as the driving force for water suction. This force is dissipated antero-posteriorly across the branchial chamber and dorso-ventrally across the gill resistance.

DISCUSSION

The scaphognathite-powered branchial pump of the lobster has been studied by simultaneous examination of the movements of the scaphognathite and the branchial pressures arising therefrom. This video-photographic analysis has allowed us to analyse the sequences of events responsible for water pumping. As in all decapod Crustacea studied, except *Corystes* (Arudpragasam & Naylor, 1966), the scaphognathite pumps work as suction devices drawing water into the branchial chambers at their lower margins between the chelae and pereiopods and expelling it through anterior excurrent channels opening just below the antennae. Photographic analysis reveals that the scaphognathite moves as a rigid blade which itself does not flex or bend, but rather effects water propulsion by changing its angle of attack during each half beat. During forward pumping the scaphognathite first closes the posterior end of the pre-branchial chamber followed by the movement of the remainder of the blade to drive the trapped water out. Each half beat ends with the scaphognathite pressed against the upper or lower margin of the pre-branchial channel, and during this brief period the negative branchial pressure moves toward zero by the back movement of water through the pump and also by water entering the gill chambers along their ventral margins.

Reversed pumping is effected by a negative angle of attack of the scaphognathite. In most cases observed in the lobster reversal beats occurred singly although more rarely they occurred in multiples. In many cases reversals occur simultaneously on both sides, but they can also occur unilaterally particularly when stimuli, such as touching the branchiostegite hairs, are applied unilaterally. In the lobster reversal beats occur only during the upstroke of the beat, whereas *Carcinus maenas* has been observed to pump backward for up to 5 sec and maintains a positive branchial pressure during this time (Hughes *et al.*, 1969) and reversed beating is the norm for *Corystes* (Arudpragasam & Naylor, 1966). In these latter two species of crabs the attack angle of the scaphognathites must necessarily remain negative during both halves of the beat and for the duration of reversed beating. That the lobster reverse beats only during the upstroke could imply that this is the preferred direction or more likely that the neuronal pathways necessary for reversed downstrokes are absent. Certainly, reversing during the upstroke takes advantage of the natural curvature (concave-upward) of the scaphognathite which provides greater pumping efficiency during this half beat. A basis for understanding the neuronal control of scaphognathite movements in decapod Crustacea has been presented by Pasztor (1968) and a further analysis of this control will be presented in a subsequent paper (Wilkins, McMahon & Colliver, in preparation).

The positive pressure produced by a reversal ends abruptly and the normal negative branchial pressure is re-established quickly. In some cases a negative overshoot is produced which lasts for 1-4 beats. The epimeral attractor muscle has been shown to contract reflexly following each reversal. This contraction has the effect of expanding the branchial chambers and also produces the observed movement of the carapace associated with reversals. Arudpragasam & Naylor (1966) observed a similar movement in *Cancer pagurus* which they felt served to regulate the size of the incurrent apertures to the branchial chambers, but which may have a functional relationship

to reversed beating as well. It is significant that the negative branchial pressure wave produced by this contraction occurs only on the side reversing and leads to the re-establishment of the previous level of negativity usually without overshoot. One may postulate the existence of pressure receptors in the gill chambers which accurately control the level of pressure attained, perhaps by a sensory feedback pathway to the epimeral attractor muscle.

The biological significance of reversal beats is not clearly understood. One proposal for their significance is that they are necessary for the proper irrigation of the posterior gills. In *Carcinus* the primary path of water movement into the branchial chambers during forward beating is through the Milne-Edwards spaces above the chelae with smaller currents entering the more posterior spaces (Arudpragasam & Naylor, 1964*a, b*; Hughes *et al.*, 1969). A water shunt around the back of gill number 9 further reduces irrigation of the posterior gill surfaces in these crabs. These authors postulated that reversed beating was necessary to irrigate the posterior gills. Indeed *C. pagarus* shows fewer reversals than *Carcinus* and takes in about half of the water posteriorly; *Carcinus* takes in most irrigation water anteriorly (Arudpragasam & Naylor, 1966). From the present study there is no evidence that reversals are necessary for the irrigation of the posterior gills of the lobster. The relative quantities of water entering each incurrent aperture along the length of the gill chambers are approximately the same. Furthermore, the epipodites of the legs which separate the gills segmentally assure that the gills of each segment are adequately irrigated. The observation of dye streams show that there are no shunts allowing water to bypass the posterior gills in the lobster.

Another proposed function of reversals is that they serve to clean detritus from gill surfaces. Arudpragasam & Naylor (1964*a, b*) found reversal rates to be correlated with an inadequate supply of water to the posterior gills in *Carcinus* which could normally arise in the presence of detritus. They also found increased rates during hypoxia and in crabs partially exposed to air. Indeed *Corystes* which normally lives buried in sand with only the anterior excurrent canals above the substrate predominantly irrigates in a reversed direction (Arudpragasam & Naylor, 1966). Both of these crab species, however, possess gill rakers which appear to clean the dorsal gill surfaces. *Homarus americanus* does not consistently show increased reversal rates during hypoxia, but does following return to water from air presumably in an attempt to clear this air from the gill chambers (B. R. McMahon & J. L. Wilkens, unpublished observations).

The evidence presented for the lobster in the present work allows yet another interpretation of the significance of reversed beating. The fringe of hairs along the bases of the branchiostegites are in a position to filter all incurrent water during forward beating. These hairs appear to have a sensory function to mechanical stimulation which may lead to reversed beating on the side stimulated. During the normal course of forward irrigation bits of detritus becoming trapped by these hairs would result in bending due to the added resistance to incurrent water flow and this could trigger the reversal beat in an attempt to wash the foreign material off.

Finally, air bubbles and milk introduced into the branchial chambers of the lobster must be considered as non-physiological stimuli which, although stimulating reversals, must be sensed by some other means than mechanoreceptive hairs. Studies on the

crayfish *Procambarus simulans*, a species not showing reversed beating, have demonstrated chemosensory modifications of both branchial irrigation and heart beat (Larimer, 1964; Ashby & Larimer, 1965). The location of these sensory receptors was postulated as inside the branchial chambers.

SUMMARY

1. The movements of the scaphognathites of the lobster, *Homarus americanus*, and the resulting thrust, were resolved in two dimensions accompanied by attack angle changes. Correlation of these scaphognathite and related body movements with branchial hydrostatic pressures during forward and reversed pumping were effected by simultaneously recording on video tape.

2. Forward pumping maintains a negative pressure within the branchial chambers of 2.5–10 mm H₂O superimposed on a maintained negative pressure relative to the outside of 0–5 mm H₂O. The scaphognathite, by assuming a negative attack angle to the water during the upstroke of the beat, produces the positive pressure of reversals.

3. Contraction of the epimeral attractor muscle appears to contribute to the re-establishment of branchial negativity following each reversal. This muscle inserts on the epimera and by contraction enlarges the branchial chamber.

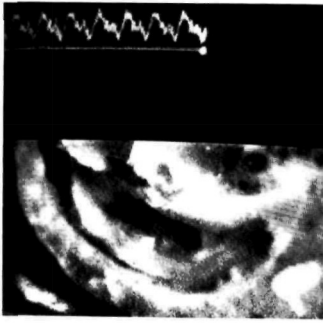
4. The role of reversals is discussed and it is concluded that their primary function is to clean foreign material from the border of hairs which filter incurrent water. Reversed beating may be a general response to all unusual stimuli to the respiratory system.

5. The pattern of water flow into and through the gill chambers was determined. The relative volume of water entering each incurrent aperture between the legs was calculated and indicated that approximately equal volumes of water entered each aperture. It is concluded that forward pumping adequately irrigates all gill surfaces.

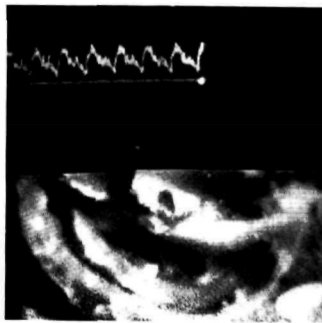
We thank Mr Gary Colliver for technical assistance throughout this study, Mr A. R. Leemburg for visual and technical consultation with video photography, and the Communications Media Department of The University of Calgary for the loan of the video equipment. This work was supported by N.R.C. grants A 5494 and A 5762 to the authors.

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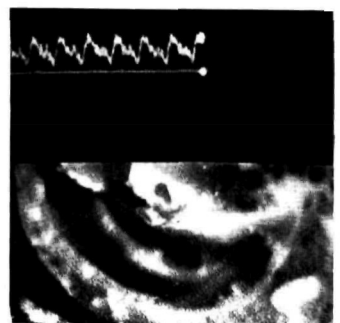
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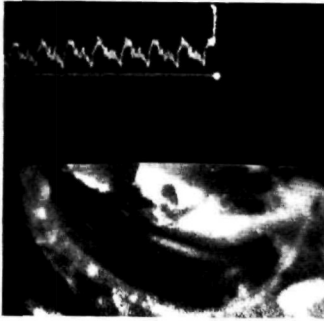
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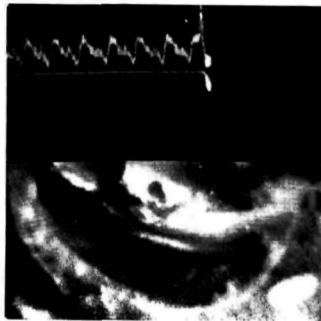
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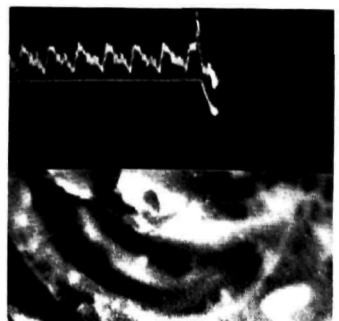
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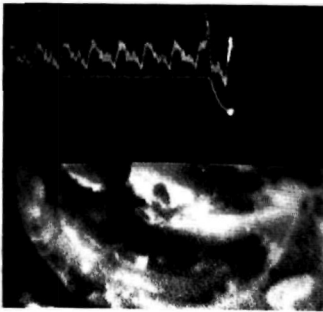
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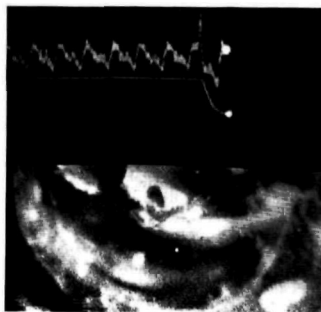
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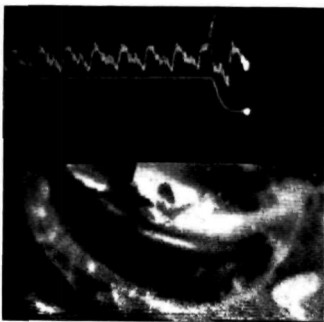
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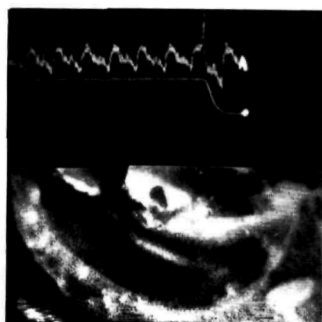
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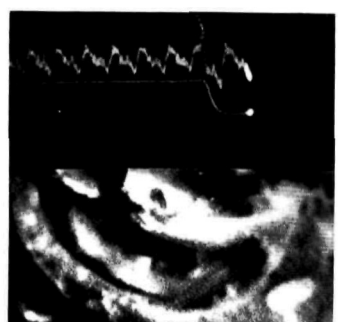
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EXPLANATION OF PLATE

Simultaneous video cinephotography of movements of the left scaphognathite as viewed through a celluloid window and oscilloscope tracings of pressure and body movement on that side. A-D show the negative attack angle assumed during a reversal beat, while E-L show the positive attack angles of the scaphognathite during forward beating in the successive down and upstroke. *a*, Hydrostatic pressure; *b*, isometric movement transducer trace; *c*, scaphognathite. Anterior end of lobster toward left.

