Water flow around a fish mimic attracts a parasitic and deters a planktonic copepod

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Fish typically evoke a flight response in copepods. The behaviour of infective stage parasitic copepods, Lepeophtheirus salmonis, and adult holoplanktonic copepods, Acartia spp., in response to the approach of a rubber fish mimic was compared. Responses were scored as attacks if the copepod moved closer to the head or escapes if it moved further from the head. The parasite attacked the mimic in 65% of its responses, whereas Acartia spp. escaped from the mimic in 87% of its responses. Lepeophtheirus salmonis escaped in sinuous routes that kept them closer to the fish, but Acartia spp. escaped in straight paths directed away from the mimic. Attacks by L. salmonis were equally frequent in the dark (68.9%) as in light (60.3%), and net-to-gross-displacement ratios under the two conditions were not significantly different. Copepod responses were evoked in regions of flow with linear strain rates greater than $0.5 \, s^{-1}$. The fish hydrodynamic signals thus serve as attractants that guide the parasite to the fish and produce an avoidance response of the holoplanktonic copepod. Thus, the same information, most likely received by the same sensors, produce ‘opposite’ reactions to the fish, suggesting the evolution of different behaviour patterns suited to the way of life of the copepod.

INTRODUCTION

Recent studies have revealed the remarkable diversity of copepod sensory abilities and behaviours. Their out-stretched antennules have a variety of sensors that monitor chemical and mechanical signals from the surrounding environment (Gresty et al., 1993; Yen et al., 1992; Lenz and Yen, 1993; Kiorboe and Visser, 1999; Lenz et al., 2000). They are usually negatively buoyant, enabling them to detect the direction of gravity, and sense the net force of acceleration of the surrounding water and their own bodies (Strickler and Bal, 1973). In some species, their eye has evolved into a complex arrangement of several lenses (Boxshall 1992; Bron and Sommerville, 1998). In response to these sensory inputs, the copepod may execute a range of behaviours such as escape, attack, or pursuit of a possible mate. The escape response of planktonic copepods and the sensory inputs required to trigger them are well documented (Schröder 1967; Haury et al., 1980; Fields and Yen, 1997; Viitasalo et al., 1998; Kiorboe et al., 1999; Buskey et al., 2002), as is the localization of motile prey by predatory copepods (Tiselius et al., 1997; Fields et al., 2002; Doall et al., 2002), and of motile mates by conspecifics (Doall et al., 1998, Weissburg et al., 1998, Yen et al., 1998, Strickler, 1998). However, the sensory-behavioural systems of parasitic copepods have received little attention. Huys and Boxshall (Huys and Boxshall, 1991) estimated that nearly half of all known species of copepods live in symbiotic relationships with other organisms. Although these spend most of their life cycle on their ‘hosts’, they usually have planktonic dispersion phases. In this phase, such copepods are usually shaped much like their holoplanktonic relatives (Kabata, 1979). At least in the case of the c. 2100 species of copepods parasitic on fish, most species have free-swimming naupliar and first
copepodid stages (G.A. Boxshall, London, personal communication), which are true members of the meroplankton. Which stimuli are required to initiate the behaviours that will eventually lead them to their hosts?

This work is focused on sensory-behavioural mechanisms that mediate the infection response of a copepod parasite of salmonids, the salmon louse *Lepeophtheirus salmonis* (Krøyer, 1837). Due to its economic importance in the salmon-farming industry, it is among the most widely studied parasitic copepods (Pike and Wadsworth, 1999). The infective stage of the salmon louse, the copepodid, carries both chemosensory aesthetascs and mechanosensory setae on its antennules (Gresty et al., 1993), indicating that both mechanical and chemical signals may be important in host-finding. Our hypothesis in this study was that the water displacements produced by the swimming host trigger the infection by the copepodid. Heuch and Karlsen (Heuch and Karlsen, 1997) found that salmon louse copepodids are sensitive to low frequency water accelerations such as those produced by a swimming fish. We surmised that reacting to the water currents around the head of the host would be advantageous as it would give the parasite a maximum amount of time to infect. Previous studies on copepodids of salmon lice (Bron et al., 1993) and other species (Boxshall, 1976; Poulin et al., 1990) similarly indicate sensitivity to hydro-mechanical signals. Recently, attraction of salmon louse copepodids to host-derived substances was also recorded in a Y-tube experiment (Bailey et al., 2006), but chemical stimulation seems to be unnecessary for initial settlement (Olsen, 2001). Other parasitic copepods, however, may depend on chemotaxis for host location (Fasten, 1913; Carton, 1968; Fraile, 1989). There is conflicting evidence as to the significance of light conditions for infection (Browman et al., 2004; Genna et al., 2005).

Here we have compared the responses of the salmon louse copepodid and the holoplanktonic copepod *Acartia* sp. to an approaching fish mimic, a rubber cast of a salmon head. We hypothesized that these two species should display marked differences in behaviour connected to the purpose of the responses, if they both rely mainly on hydro-mechanical cues for host or predator detection. The parasite should approach the fish as it could be a host, whereas the non-parasite should try to move away as the fish could be a predator.

**METHOD**

**Study animals**

*Acartia* sp. are holoplanktonic, omnivorous calanoid copepods. The life cycle includes six naupliar and five copepodite stages before the adult (Gonover, 1956). Specimens were collected with a plankton net in Stony Brook Harbor, Long Island, NY, and brought to the laboratory at State University of New York at Stony Brook in insulated containers. Adults were sorted out and used in experiments. At the time of collection there was a mixture of *Acartia tonsa* and *Acartia hudsonica* in the plankton, and no attempt was made to separate these morphologically very similar species, which attain a length of c. 1.0 mm. These are henceforth referred to as one 'species', *Acartia*. The sorted animals were kept in glass jars with filtered (1 μm) water from Stony Brook Harbor in an incubator at 12°C. Animals were not fed.

*Lepeophtheirus salmonis* is a Caligid copepod which parasitize salmonid fish in salt water in the Northern Hemisphere (Kabata, 1979). The life cycle includes ten stages, three of which are pelagic. The third of these is the infective copepodid. This c. 0.7-mm long stage must attach to a host within 10 days at 10°C, or it will die (Johnson and Albright, 1991). On the fish, four sessile caliminus and two preadult stages follow before the moult to adult. Egg sacs were collected from adult female parasites on Atlantic salmon (*Salmo salar* L.) from salmon farms in Maine, USA. Sacs were sent by air in insulated sea water-filled containers, and were treated as described by Heuch and coworkers (Heuch et al., 1995) until eggs hatched. Nauplii were kept in glass jars in an incubator at 12°C. Moulting to copepodid was monitored, and only copepodids between 2 and 6 days old were used in experiments. Filtered (1 μm) sea water of salinity 33 was used for rearing and experiments involving parasites.

**Host mimic**

Atlantic salmon (*S. salar* L.) smolts are highly susceptible to *L. salmonis* (Grimnes and Jakobsen, 1996; Pike and Wadsworth, 1999) and therefore were used as model fish. A mould of the head of a frozen 15-cm long Atlantic salmon smolt was made by suspending the fish head-first into a beaker, and filling the latter with plaster of Paris. The fish was removed when the plaster had set, and the mould was gently brushed clean when dry. White silicone rubber (Wacker GmbH., Germany) was subsequently used to make casts. Casts were approximately 4 cm long, 1.5 cm at the widest and 3 cm at the highest point (behind the gills). A glass rod (manual propulsion experiment) or stainless steel threaded rod (piston propulsion experiments) was inserted in the setting silicone in the back of the head to enable attachment to propulsion equipment without disturbing the anterior flow around the head.
Experimental presentation of fish head

**Manual propulsion**

In a first series of observations, the head was moved manually and responses were recorded and analyzed in three dimensions. The head cast was propelled horizontally by pushing a rod inserted through a sealed (by two o-rings) centrally located entrance on one side of the 15 × 15 × 15 cm clear Perspex experimental tank. Two Pulnix CCD cameras, mounted perpendicularly on a standing 1 × 1 m aluminum frame surrounding the tank, were used to record copepod behaviour in three dimensions. Each camera, recording at 30 frames s⁻¹ (60 half-frames, maximum temporal resolution of 16.7 ms), was connected to a VCR through a frame counter. The entire observational system was set up in a walk-in environmental chamber to maintain ambient temperatures around 12°C.

The tank was illuminated by a white 100-W light bulb and two white fiber optic lights. Thirty animals of one species were introduced in the tank at a time. They were allowed to acclimatize for >30 min before the head was moved. The light bulb was initially switched off, and the fiber optic lights shone at the tip of the head to concentrate the copepods in the path of travel. When sufficient animals had gathered in the middle of the tank, the bulb was switched on in order to produce a bright and uniform light field, and the head advanced soon after. There was a pause of c. 1 min between pushes to avoid habituation (Heuch and Karlsen, 1997).

**Piston-driven propulsion**

In a second series of experiments, we standardized the movement of the fish mimic and the accompanying hydrodynamic signal using a piston-driven propulsion system. The cast of the salmon smolt head was mounted on a 0.2 × 3 × 10 cm stainless steel blade, which entered the 20 × 20 × 25 cm clear Perspex experimental tank from above. The leading edge of the blade, which rose up behind the head, was rounded off to reduce turbulence. The head was attached to the edge of the blade 10 cm down in the tank. The top end of the blade was secured onto an aluminum block, which was moved by two gas-driven pistons. The pistons lay side by side in one rectangular casing, which was attached to a 1-cm thick aluminum sheet over the tank. This ‘ceiling’ was supported by four legs connected to the 2-cm aluminium sheet ‘floor’. The aluminium housing and all other equipment were secured to a Newport air-damped table.

To visualize the copepods, an infrared (IR) laser was used to create a Schlieren optical pathway that provided a two-dimensional view of almost the entire vessel (Strickler, 1998). To control the presence of visible light, the entire table was walled in with double sheets of black plastic. Recordings were made with and without visible light to determine if the infection response of *Lepeophtheirus* is mediated by vision. *Acartia* responses were examined in the dark only. For the light experiments, a white fiber optic light was turned on in addition to the IR Schlieren optical path. Events were video-recorded with one Pulnix CCD camera connected to a VCR through a frame counter. Turning the piston casing 90° allowed switching between head-on and side views of the fish mimic.

All experiments using this observation system were performed at room temperature with trajectory analyses documented only on the actively swimming individuals. About 50–100 animals of one species were used at a time in the tank, and these copepods were allowed to acclimatize to room temperature (20°C) 2–4 h before being used in experiments.

Analysis of fish head speeds

The speed of the advancing head was calculated from instantaneous velocities measured every video field, i.e. every 16.7 ms. In the manual propulsion experiments, head speeds varied considerably both within and between events (Table I). Event durations were also highly variable. The median head speed therefore was calculated for each of the events where responses were observed, and the mean of these medians subsequently was calculated to indicate signal strength for each

<table>
<thead>
<tr>
<th>Type of experiment</th>
<th>Species</th>
<th>Mean velocity (mm s⁻¹)</th>
<th>SE (mm s⁻¹)</th>
<th>Mean duration (s)</th>
<th>SE (s)</th>
<th>n (events)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manual propulsion</td>
<td><em>Acartia</em> spp.</td>
<td>63.6 ± 10.5</td>
<td>0.85 ± 0.070</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>L. salmonis</em></td>
<td>47.4 ± 8.9</td>
<td>1.30 ± 0.189</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Piston propulsion</td>
<td><em>Acartia</em> spp.</td>
<td>117.7 ± 4.3</td>
<td>0.76 ± 0.003</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>L. salmonis</em></td>
<td>115.6 ± 4.5</td>
<td>0.75 ± 0.003</td>
<td>10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For the manual propulsion experiments, the mean was calculated from the median head speed of each event (i.e. each head advance). For the piston propulsion experiments, the mean was calculated using repeated measures statistics (see Methods for details).
species of copepod. The median speeds of the head from start to stop in the manual propulsion *L. salmonis* experiments events ranged from 13.1 to 108.5 mm s\(^{-1}\), giving a mean speed of 47.4 mm s\(^{-1}\) (Table I). In the *Acartia* experiments, the median speed during events ranged from 37.4 to 125.2 mm s\(^{-1}\), giving an average head speed of 63.6 mm s\(^{-1}\). The means of median head speeds were not significantly different between the two species (*t*-test, *P* = 0.26).

The duration of the piston propulsion events were equal, hence a more sophisticated analysis of head movements was possible. As a speed record at a given time is correlated with the speed immediately preceding this record, a repeated measurements analysis was employed. The correlations were incorporated into the statistical model using a first-order autoregressive covariance structure (AR (1)), and type 3 testing was employed (Littell *et al.*, 1996). In the piston propulsion experiments, the movement of the rubber head had three distinct phases: First acceleration for c. 50 ms, then ‘cruising speed’ at 120–230 mm s\(^{-1}\) for c. 700 ms, and a deceleration lasting about 50 ms (Fig. 1). During the *Acartia* experiments, the mean speed for the entire movement was 117.7 mm s\(^{-1}\) (± 4.3 SE), and for *L. salmonis* 115.6 (± 4.5 SE). These mean speeds were not significantly different (*F* = 0.23, *P* = 0.64, Table I). Variations in speed during the cruising phase followed a wave pattern, which was very similar between events. This phase could again be divided into two parts, where the former had larger oscillations than the latter (Fig. 1). During the first half, the velocity fluctuated in the order of ±40 mm s\(^{-1}\) at a frequency of c. 10 Hz, around a mean of 163.6 ± 3.7 (SE, *n* = 20) mm s\(^{-1}\). In the second half, the mean velocity was 159.8 ± 3.7 (SE, *n* = 20) mm s\(^{-1}\).

**Analysis of water flow velocity**

Water velocity measurements were performed by particle image velocimetry technique (Westerweel, 1997; Raffel *et al.*, 1998, Yen *et al.*, 2003). The technique determines the velocity field of a fluid by measuring the displacement of very small suspended particles over a brief time interval. Combining the displacement estimate with the image magnification and time interval between laser pulses yielded the local velocity. Derivative properties, such as the strain rate, then were calculated directly from the velocity field using a central finite-difference scheme.

**Analysis of copepod trajectories**

Each event (push or head advance) was recorded digitally from the analogue VCR tapes to a computer using a video-capture card. The kinematics of the swimming trajectories were analyzed frame by frame in the motion analysis module of Optimas software (Media Cybernetics, Silver Springs, MD, USA).

Initially, 15 obvious *Acartia* and 15 obvious *Lepeophtheirus* responses to the moving head were tracked in 2D; and gross displacement, mean and maximum swimming velocity during the response were calculated. Normal swimming behaviour then was characterized by tracking the same copepods prior to their response, and

![Fig. 1. Mean speed of the piston-driven salmon head mimic (±SE) calculated at each 0.0167-s interval of travel. *N* = 20 (10 *Acartia* spp. and 10 *Lepeophtheirus salmonis* experiments) at all but the three last points, which were *n* = 13, 4 and 1, respectively.](image-url)
estimating the same parameters, over the same time interval as the response duration. This permitted a pairwise comparison of swimming behaviour, controlling for other factors that may have influenced behaviour such as proximity to walls and physical condition of copepod. For normal swimming, each copepod was tracked for 3 s, and the gross displacement and mean velocity were computed over every sub-interval equal to the duration of the response within those 3 s. For instance, if the response was 10 frames long, normal swimming was tracked prior to response for 3 s (i.e. 90 frames), and then the gross displacement and mean velocity were computed for every 10 frame interval within those 3 s. All these gross displacements and mean velocities measured then were averaged. Maximum velocity during normal swimming was calculated as the maximum instantaneous velocity (i.e. 1 video field) during the 3 s of swimming prior to response. Pair-wise t-tests showed that the values of the three parameters were significantly elevated during the response \( (P < 0.0005) \). For the remainder of the analyses, a response was defined as swimming which exceeded twice (a) the maximum values of gross displacement, (b) mean velocity and (c) maximum normal swim velocity found during normal swimming of the 15 animals of each species. Threshold values thus were set at 2.8 mm gross displacement, 7.6 mm s\(^{-1}\) mean velocity and 85.5 mm s\(^{-1}\) max velocity for *Acartia*, and 6.0 mm gross displacement, 13.9 mm s\(^{-1}\) mean velocity and 81.9 mm s\(^{-1}\) max velocity for *L. salmonis*.

Within the field of view there were some animals which did not react to the moving head, and at the resolution of our set-up, these were difficult to separate from air bubbles and particles of dust. Enumeration of passive animals or particles therefore was not carried out, and the analysis was focused on the responding animals.

For each event, we observed from zero to up to nine responses to the fish mimic. Responses were categorized as attack or escape, based solely on whether the copepods moved closer to (i.e. attack) or further from (i.e. escape) the fish mimic. Analyses of net-to-gross-displacement ratios (NGDR) and reactive distances were performed using the 2D response trajectories recorded during the piston propulsion experiments. Data from both dark and light experiments were used. The net displacement and gross displacement were computed for each individual response, and means and SD of these are reported for both reaction types for both species. The NGDR was calculated for each response. The distribution of the NGDRs was strongly skewed and were therefore arcsine-transformed before t-tests were employed. Reported NGDR values were back-transformed from the calculated means and deviations.

For reactive distances, only responses where the copepod was clearly visible at the start of the response, were included in the analyses. The average swimming speeds reported were calculated from the \( x-y-z \) coordinates of response trajectories recorded in the manual advance experiments. Swimming speeds were not adjusted for the water movement created by the fish head, as these complex displacements were not simultaneously recorded. Data distributions were plotted as normal quantiles to assess normality, and differences were tested by t-tests if data were approximately normal.

**RESULTS**

In the manual propulsion set-up, 29 events (head pushes) including 48 individual copepod responses were recorded for *L. salmonis* (Table II). Ten responses were tracked in...
3D. For *Acartia*, 12 events were recorded and 21 responses were analyzed, of which 11 were tracked in 3D. Using the piston propulsion set-up, a total of 184 events including 253 responses were recorded and analyzed.

**Attacks and escapes**

*Acartia* and *L. salmonis* displayed striking differences in swimming behaviour (Figs 2 and 3, Table III). When approached by the fish mimic, 65% of the *L. salmonis* responses were attacks; only 13% of *Acartia* responses were attacks (Table II). Typically, *L. salmonis* copepodids reacted with rapid sinuous displacements without pauses when the head approached (Figs 2a and b and 3a), while *Acartia* swam in straight jumps, often with brief pauses in movement (Figs 2c and 3b). This resulted in significantly lower NGDRs both during attacks (t-test, \( P = 0.031 \)) and escapes (t-test, \( P < 0.0001 \), Table III) for *L. salmonis*. The straight short jumps of *Acartia* translated them a shorter total distance in a direction farther from the center of the head path than *L. salmonis* during both attacks and escapes (Table III). Parasite attacks tracked in 3D had a mean speed of 94.81 \( \pm 4.01 \) mm s\(^{-1}\) (SE, \( n = 8 \)), whereas *Acartia* average jump speed (jump defined as movements \( > 1 \) mm in 3D) was 130.21 mm s\(^{-1}\) (\( \pm 10.83 \) SE, \( n = 11 \)). Maximum recorded speed in a single escape jump was 332.5 mm s\(^{-1}\).

When in the path, or very close to the path, of the approaching fish mimic, the parasitic copepodids often initially swam away and then circled around to attack the mimic as it passed (Fig. 3a). These ‘circle attacks’ were seen in all experiments and under all light conditions. In some circle attacks, the parasite apparently initially was pushed out by the flow. It then reacted by swimming away and subsequently back, often making contact with the head. When they were further out from the mimic’s path, the parasites typically would follow approaches oriented more directly at the mimic (Fig. 2a and b). There were few *Acartia* ‘attacks’, and these generally followed straight paths (Fig. 2c). *Acartia* was never seen in contact with the moving head. Escape paths by *Lepeophtheirus* often curved back toward the head near the end of the response (Fig. 2a and b) or had multiple loops or turns.

**Behavioural sensitivity**

Reactive distance, or the distance between the copepod and the fish mimic at the initiation of response, was measured for each response in both 2D views of the piston propulsion experiments. In the head-on view (i.e.* Z–Y* plane), reactive distances were measured to the centre of the fish mimic, a point which marked the

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**Fig. 2.** Two-dimensional trajectory of (A) 45 responses of *Lepeophtheirus salmonis* in dark, (B) 63 reactions of *L. salmonis* in light, and (C) 61 responses of *Acartia* sp. in dark when stimulated by an approaching rubber salmon head (head-on view). Each trajectory is shown as a series of connecting vectors. Each vector shows the magnitude (length) and direction of velocity in a 1/60-s interval. Reactions moving toward the head (i.e. attacks) are colour coded red. Reactions moving away from the head (i.e. escapes) are colour coded blue.
central axis of the fish mimic’s path (i.e. X-axis). Thus, reactive distances in the head-on view expressed the tangential distance to the center of the mimic’s path. Reactive distances in head-on view averaged 26.28 mm (±10.13 SD, \(n = 47\)) for \(L.\) salmonis, and 27.99 mm (±16.34 SD, \(n = 61\)) for \(Acartia\) (Fig. 4a), and these were not significantly different (t-test, \(P = 0.54\)). In both species of copepod, there were responses to the fish head both before it reached them, and as it passed them (Fig 4b). In side view, reactive distances were measured to the tip of the fish mimic’s nose and averaged 25.71 mm (±19.26 SD, \(n = 13\)) for \(L.\) salmonis and...
Table III: Quantitative analysis of displacements of Acartia spp. and L. salmonis during responses to an approaching rubber salmon head

<table>
<thead>
<tr>
<th>Species</th>
<th>Behaviour</th>
<th>Net displacement ± SD (mm)</th>
<th>Gross displacement ± SD (mm)</th>
<th>Net to gross displacement ratio ± SD</th>
<th>Time between start of head movement and start of response ± SD (ms)</th>
<th>Final distance from head path axis (mm) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acartia</td>
<td>Attack</td>
<td>13.35 ± 11.0</td>
<td>15.14 ± 11.1</td>
<td>0.92 ± 0.3</td>
<td>411.11 ± 150.9</td>
<td>24.02 ± 15.6</td>
</tr>
<tr>
<td></td>
<td>Escape</td>
<td>19.52 ± 14.0</td>
<td>21.4 ± 14.7</td>
<td>0.91 ± 0.2</td>
<td>295.51 ± 179.3</td>
<td>41.45 ± 14.8</td>
</tr>
<tr>
<td>L. salmonis</td>
<td>Attack</td>
<td>17.20 ± 10.9</td>
<td>20.73 ± 15.9</td>
<td>0.74 ± 0.37</td>
<td>240.58 ± 182.6</td>
<td>16.34 ± 6.3*</td>
</tr>
<tr>
<td></td>
<td>Escape</td>
<td>23.55 ± 10.7</td>
<td>35.32 ± 10.8</td>
<td>0.75 ± 0.4</td>
<td>212.82 ± 165.5</td>
<td>31.40 ± 9.3*</td>
</tr>
</tbody>
</table>

Times and distances were calculated from 2D head-on view piston propulsion experiments. Both were computed for each individual response, and summary statistics computed. The NGDRs were calculated for each response, and were arcsine-transformed. Reported NGDR values were back-transformed from the calculated means and deviations.

\*n = 9, \*n = 52, \*n = 69, \*n = 29, \*n = 39, \*n = 16.

Copepod responses to the approach of the fish head were observed at all stages of the fish mimic velocity profile (Fig. 1) in the piston propulsion experiments. The mean time between start of head motion and start of copepod response was shorter for the parasite than for Acartia (Table III). On average, L. salmonis attacks were initiated 240 ms after the head had started, compared to 411 ms for Acartia. Parasite escapes occurred on average 212 ms after the head started moving, compared to 295 ms for Acartia. Most responses were initiated during the ‘cruising’ phase of head movement. Only 13.9% of L. salmonis responses and 14.8% of Acartia responses in the dark experiments started when the head was in the initial accelerating phase, i.e. during the first 0.05 s of head motion (Fig. 1).

The effect of light

All events in the manual advance experiments were recorded using white light to illuminate the tank. The concentrated beam of the fiber optic light reflected off the white silicone fish head, and conveniently attracted copepods into the path of travel of the mimic. In the piston advance set-up, responses of both copepod species were recorded using infrared light only, to see if this would change copepod behaviour. However, a similar proportion of L. salmonis responses were attacks in the dark (IR) (68.9%) and in light (IRW) (60.3%, Fig. 2a and b). The proportions were not significantly different (Chi2-test, 0.75 < P < 0.5). The NGDRs for L. salmonis were not significantly different in light (n = 63) and dark (n = 45) (t-test on arcsine-transformed data, P = 0.35). The response times (interval from start of head movement and start of copepod response) were not significantly different in light (mean 219.8 ms) and dark (mean 245.9 ms, t-test, P = 0.45).
Flow field around the fish head

Flow speeds, and the intensity of the linear strain rate in the direction of the flow, increased strongly close to the piston-driven fish head (Fig. 5). At distances closer than 2.5 cm from the head, the flow velocity was greater than 0.5 cm s⁻¹, with strain rates increasing above 0.5 s⁻¹ to 8 s⁻¹ (Fig 6).

DISCUSSION

Fish are primary predators on copepods, and many authors have described copepod flight responses (Schröder, 1967; Haury et al., 1980; Fields and Yen, 1997; Viitasalo et al., 1998; Buskey et al., 2002). It is more difficult to evoke an attractive response in copepods, and there are fewer examples of signals that increase the encounter rate between aquatic organisms. Fields and Yen (Fields and Yen, 2002) and Tiselius and coworkers (Tiselius et al., 1997) have documented such an increase for copepod attack responses, and Doall et al. (Doall et al., 1998) showed how a male copepod is attracted by sex-specific female behaviour. Here we have presented 2D and 3D trajectories of the approach of a copepod parasite to a fish host. The L. salmonis response is qualitatively and quantitatively different from the response of the similarly sized holoplanktonic Acartia sp. to the same stimulus. The parasitic copepod attacks instead of fleeing. The fish signals apparently serve as attractants that guide the parasite to the fish. Thus, the same information, most likely received by the same sensors, produce ‘opposite’ reactions to the fish, suggesting the evolution of different behaviour patterns suited to the way of life of the copepod.

The results strongly support the hypothesis that the infection response of the salmon louse copepodid is triggered by hydro-mechanical signals, as no fish-related compounds were present in the tanks, and responses took place in the dark. In the following discussion, the behaviours of the two species are compared, and relevant triggers for the salmon louse infection response are discussed. Finally, the place of the observed behaviour of the parasite in the sequence of events leading to fish infection is assessed.

Differences in behaviour between parasitic and holoplanktonic copepods

The differences in behaviour between the copepod species were consistent through the experiments, in light and in darkness. Acartia usually evaded the head in a short series of straight high-speed jumps, propelling them away from the path of travel of the head. These escape responses were similar to previously reported escape behaviour in this genus (Kiørboe et al., 1999; Suchman, 2000). Jump speeds were higher than recorded in A. hudsonica escaping scyphomedusae (Suchman, 2000), possibly reflecting the faster approach of the fish head. The escape speeds measured in the present experiments are comparable with the data of Buskey et al. (Buskey et al., 2002), who recently recorded escape speeds using high-resolution video of 1000 frames s⁻¹. They calculated a maximum speed of 656 mm s⁻¹ for Acartia tonsa, and mean speeds over entire escape responses from 120 to >570 mm s⁻¹. The maximum speed recorded in our 3D experiments was 332.5 mm s⁻¹, while mean escape speed was 130 mm s⁻¹ for Acartia sp. The maximum swimming speed may have been influenced by the water displaced by the moving head, but average speeds would be based on records from areas both remote from and close to the head path, and would thus represent better estimates of natural speeds. As reported speeds are well within the ranges previously published for this genus, we conclude that Acartia sp. swim speeds in our experiments were comparable to natural speeds.

Two general types of parasite attacks were apparent in our experiments. If the copepodids were in or near the path of the advancing fish they most frequently swam away first, and subsequently turned towards the head, often making contact with it (Fig. 3a). Such circle attacks were never performed by Acartia sp., and these copepodids never touched the head. For the parasite, which also may be ingested by planktivorous fish, the circle swimming behaviour appears to both remove it from the mouth area of the fish, and guide it towards the fish body. Several authors have noted that salmon louse copepodids appear to have a preference for host fins (Bron et al., 1991, 1993; Birkeland, 1996; Tucker et al., 2002). To the extent that the fish fins are folded out during the copepodid infection response, they are likely to be in the path of copepodids circling the fish body at close range.

In the 2D experiments, the water temperature was c. 20°C, presumably exceeding the natural habitat temperature of L. salmonis. However, the animals appeared vital, and there was no sign of differences between the responses at this temperature and in the 3D experiments at 12°C. Furthermore, a similar proportion of them attacked and escaped at the two temperatures (Table II).

In response to water disturbances, the copepodids of L. salmonis (Bron et al., 1993), L. procatoralis (Boxshall, 1976) and Salmincola edwardsii (Poulin et al., 1990) swim in characteristic tight spirals. L. salmonis copepodids also
Fig. 5. (A) Flow velocity magnitudes, determined from a single instantaneous data set of the velocity gradient, visualized around the moving salmon head silicone cast. Velocity is \(\sqrt{U^2 + V^2}\), where \(U\) is the flow in the \(X\)-direction parallel to body axis of fish, and \(V\) is the flow in the \(Y\)-direction perpendicular to the body axis of the fish. Linear transparency mapping was applied to velocity data between 0 cm s\(^{-1}\) and 1.5 cm s\(^{-1}\); 0 cm s\(^{-1}\) are transparent, with increasing velocities becoming increasingly opaque up to 1.5 cm s\(^{-1}\). After 1.5 cm s\(^{-1}\), all velocity data are opaque. Scale is in cm s\(^{-1}\). (B) Linear strain rates parallel to the flow (\(XX\)-direction) visualized around the fish head. Flows were determined from a single instantaneous data set of a planar-view of the velocity gradient parallel to flow bisecting fish head. Linear transparency was applied between −1.75 and 1.75 1 s\(^{-1}\). Scale is in 1 s\(^{-1}\). The head was driven by pistons at 16 cm s\(^{-1}\).
moved in sinuous paths when escaping from the advancing fish mimic in the present study. While this behaviour could be regarded as an avoidance response, as this species would regard approaching fish as possible predators, it can also be considered part of an approach, since other copepods show sinuous swimming trajectories associated with search behaviour (Katona, 1973; Uchima and Murano, 1988; Doall et al., 1998).

**Triggers for copepod responses**

Copepods of the genus *Acartia* are known for their ability to detect hydro-mechanical signals (Yen and Fields, 1992; Fields and Yen, 1997; Kiorboe et al., 1999, Suchman, 2000; Buskey et al., 2002). In the present study, the *Acartia* spp. responded to the moving head with characteristic straight jumps which brought them out of the path of the head. This happened both in light and darkness, and there were no chemical signals present in the experimental chamber. We assume therefore that the responses were triggered by hydro-mechanical signals. Similarly, both salmon lice (Bron et al., 1993; Heuch and Karlsen, 1997) and other parasitic copepodids are sensitive to hydro-mechanical signals (Poulin et al., 1990). Salmon louse copepodids are sensitive to low frequency water accelerations such as those produced by a swimming fish, possibly because they are slightly denser than sea water (Heuch and Karlsen, 1997) such that flow acceleration on scales larger than the animal may induce bending of setae (Strickler and Bal, 1973; Yen and Fields, 1992). The ability to sense this hydro-mechanical stimulus has also been demonstrated for the holoplanktonic copepod *Cyclops abyssorum* (Schröder, 1967), and is thus not unique for parasites.

When *Acartia* spp. adults and *L. salmonis* copepodids first reacted to the fish head, they were equally distant from its nose, both in side view and head-on view. They were also equally sensitive to the initial acceleration of the fish head. This could indicate that they are equally sensitive to hydro-mechanical signals such as the velocity or acceleration difference between their bodies and the surrounding water (Kiorboe et al., 1999). However, there is evidence to suggest that the two copepods respond to different components of the velocity differences in the flow. *Acartia tonsa*, at least, is very sensitive to deformation rate (Kiorboe et al., 1999), whereas acceleration alone gives a strong response in *L. salmonis* copepodids (Heuch and Karlsen, 1997). Immediately in front of an approaching fish, the rotational component of the velocity gradient is zero, and, theoretically, the signal which is most likely to be perceived by a 1-mm copepod is deformation rate (Kiorboe and Visser, 1999). The sensitivity to deformation rate in *L. salmonis* copepodids is not known, but it remains possible that this signal may also be used in host detection. The analyses of the flow field suggest that the hydro-mechanical signals increase in strength dramatically at distances closer than 2.5 cm from the moving mimic. Flow speeds at this location are greater than 0.5 cm s\(^{-1}\), and linear strain rates parallel to the fish head movement are >0.5 s\(^{-1}\). Studies by others (Fields and Yen, 1997, Kiorboe et al., 1999) have also showed that threshold strain rates greater than 0.5 s\(^{-1}\) produce escape responses in *Acartia* species, and a threshold deformation rate of

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**Fig. 6.** Profiles along a direct approach toward the nose of the salmon head showing the absolute values of the flow speeds and linear strain of the flow generated around the piston-driven fish mimic. Linear strain rates perpendicular to the head movement were close to zero and the shear strain rates were <25% of the maximum strain rates and therefore were not plotted here. The head speed was 16 cm s\(^{-1}\).
0.4 s⁻¹ has been reported for Calanus finmarchicus (Haury et al., 1980). Further analyses are recommended to discern which components of the deformation rate serve as the hydrodynamic signal, and whether there are species-specific responses to different flow structures.

**Host-finding in L. salmonis**

Host-finding in parasitic copepods of fish may be divided into three phases. First, the copepod must be in the same waters as its host. Second, it must be able to detect the host once this is in within swimming distance and execute behaviours which will bring it in contact with the fish. This is equivalent to what we here call the infection response. Third, the copepod must be able to recognize the correct host once it has made contact. *Lepeophtheirus salmonis* is a parasite specific to the salt-water stages of the fish genera *Salmo, Onchorhynchus* and *Salvelinus* (Kabata, 1979). The nature of this specificity may be a result of processes occurring in all three phases of host-finding. If the infection response is triggered by unspecific deformation of water around the fish as suggested by our results, it is likely that any free-swimming fish with a similar shape and swimming mode as salmonids could be infected by the copepods initially.

Laboratory experiments have confirmed that salmon louse copepods may infect other fish species, but they abandon these within hours of initial contact (Olsen, 2001). This author monitored salmon lice copepodid and chalimus numbers on laboratory infested saithe (*Pollachius virens* L.) and Atlantic salmon at intervals until 265 days post-infection (PI). He found that the copepodids attached to both fish species initially, but had left nearly all saithe hosts 50 h PI. The decline in numbers of copepodids on saithe was faster in a mixed population of saithe and salmon than in a population of saithe only, indicating a transfer of parasites from saithe to salmon (Olsen, 2001). The failure of Bron et al. (Bron et al., 1993) to induce salmon louse copepodid settlement on saithe may be attributed to an unnatural swimming pattern of the fish due to the small size of the experimental tank (1 L).

Other sensory modalities which may be involved in host-finding include vision and chemotaxis. The salmon louse copepod has a complex eye equipped with three lenses (Boxshall, 1992; Bron and Sommerville, 1998), suggesting the possibility of light or shadow as attractant or guide in the infection process. Several studies have shown that the salmon louse copepod is positively phototactic (Johannessen, 1975; Bron et al., 1993; Heuch, 1995; Flamarique et al., 2000), but comprehensive laboratory experiments by several workers (Bron et al., 1993; Flamarique et al., 2000) show no response to shadows, as found for the closely related *Caligus elongatus* (Hogans and Trudeau, 1989). Recently, it was demonstrated that salmon lice copepodid infection success in large tanks was not influenced by different qualities of light; neither polarized nor UV-A light produced higher infections than darkness (Browman et al., 2004). Successful laboratory infections with salmon louse copepodids in complete darkness have also previously been reported (Johnson and Albright, 1991; Bron et al., 1993). However, Genna et al. (Genna et al., 2005) stated that a light intensity of 300 lx produced higher settlement of copepodids than 800 lx or 10 lx in flume infections of Atlantic salmon smolts. The present work shows that copepodid behaviours in light and dark are not significantly different, and thus supports the conclusion that light does not appear to be required for infection.

The present experiments show that copepodids will attempt to infect a rubber head devoid of fish chemicals, in an environment without host-derived substances. The copepodid thus appears able to detect its host, i.e. carry out Phase 2 of host-finding, without chemical cues. Other authors have earlier failed to induce attraction of copepodids to host-derived substances (Bron et al., 1993), but Bailey et al. recently observed attraction to ‘salmon-conditioned’ water in a Y-tube experiment (Bailey et al., 2006). On settlement, the salmon louse copepodid behaves as it inspects the host, repeatedly prodding the fish skin and moving its antennules over it before injecting the anchoring filament (Bron et al., 1991). It has been suggested that the two chemosensory aesthetascs on each antennule (Gresty et al., 1993) may be used in host identification in this phase (Boxshall, 1976; Bron et al., 1991, 1993). It is likely that these sensors are active before host contact, but their function for the planktonic copepodid in nature is not known. Contact chemical host identification, however, has earlier been demonstrated for infective copepodids of the parasitic copepods *L. pectoralis* (Boxshall, 1976) and *Lernaeocricetus stagnalis* (Anstensrud and Schram, 1988). Furthermore, host-derived chemicals appear to be involved in host identification in the copepodids of *S. edwardsii* (Fasten, 1913), *Sabelliphilus sarsi* (Carton, 1968), and *Caligus minimus* Otto (Fraile, 1989). Apparently, the two types of sensory elements on the copepodid antennules serve different functions: the mechanosensory setae are used in guiding the copepodid to a prospective host (Phase 2), and the aesthetascs provide information about the identity of the host once the copepod has settled (Phase 3). At the adult stage in the louse life cycle, the aesthetascs appear to be used in reattachment and mating (Devine et al., 2000; Ingvardsdottir et al., 2002a, b).

Whatever the hydrodynamic signal, very different responses from the two copepod species were observed.
For *L. salmonis*, the response could be an active attack or a suppression of the escape response, leaving the copepod to follow the flow like a passive particle tracer until it is close enough to attach itself. This distinct difference in the responses of the copepods to flow thus enables the approach of the parasite to its fish host. In summary, the present work strongly supports the hypothesis (Heuch and Karlsen, 1997) that hydro-mechanical signals in the high velocity gradient around the fish head trigger the rapid swimming and subsequent attachment to the host by the salmon louse copepodid.

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