Response of *Neobenedenia girellae* (Monogenea) oncomiracidia to brightness and black-and-white contrast

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SUMMARY

Neobenedenia girellae, a capsalid monogenean, is a significant pathogen due to both its ability to cause high mortality in fishes and its low host specificity. Established control methods have both advantages and disadvantages. Biological control measures with no unfavourable effects on the environment should be incorporated into the control strategy. The response of *N. girellae* oncomiracidia to brightness and black-and-white contrast was investigated to search for an alternative approach of disease prevention or control. Japanese flounder, *Paralichthys olivaceus* (Paralichthyidae), were exposed to oncomiracidia in an aquarium divided into areas of different brightness (~1·3, 41·3 and 138·0 lux). The number of parasites on the fish group reared in 138·0 lux was significantly higher than on those reared in the lower brightness levels. Thus, the fish tended to be more vulnerable to infection by *N. girellae* under brighter conditions. Challenge trials using host fish mucus and whole live fish were established to detect the response by oncomiracidia to black-and-white contrast on a white *versus* a black background. Markedly more *N. girellae* oncomiracidia attached to black-painted areas and dark-coloured fish (normal spotted halibut, *Verasper variegatus* (Pleuronectidae) compared with white-painted areas and light-coloured fish (mal-coloured *V. variegatus*) on a white-coloured background. On a black-coloured background, more *N. girellae* oncomiracidia tended to attach to white-painted areas and light-coloured fish. Thus, black-and-white contrast is considered important for host finding by *N. girellae* oncomiracidia. The simplicity of the positive phototactic behaviour and the response to black-and-white contrast may lead to the development of a simple, practical and inexpensive method to control *N. girellae* outbreaks.

Key words: *Neobenedenia girellae*, oncomiracidium, phototaxis, black-and-white contrast.

INTRODUCTION

Many parasites are serious pathogens in intensive aquaculture in Japan (Ogawa and Yokoyama, 1998). *Neobenedenia girellae*, a capsalid monogenean, is one of them because of its ability to cause high mortality in host fishes (Ogawa *et al*. 1995) and its broad host specificity (Bondad-Reantaso *et al*. 1995). Some commercially important, cultured fishes, such as yellowtail, *Seriola quinqueradiata* (Carangidae), amberjack, *Seriola dumerili* (Carangidae), tiger puffer, *Takifugu rubripes* (Tetraodontidae), Japanese flounder, *Paralichthys olivaceus* (Paralichthyidae), and spotted halibut, *Verasper variegatus* (Pleuronectidae), become infected with this monogenean (Ogawa and Yokoyama, 1998; Hirazawa *et al*. 2004).

Bondad-Reantaso *et al*. (1995) described the life-cycle of this parasite. At a water temperature of 25 °C, free-swimming oncomiracidia (body length: ~200 μm) hatch from eggs after 4 days, attach predominantly to the fins and then migrate from the fins to the skin surface as they grow. The maturation of *N. girellae* (body length: ~2·1 mm) takes 10 days from larval attachment to the host. The life-span of *N. girellae* oncomiracidia has not yet been investigated, but the typical life-span of a monogenean oncomiracidium is usually 24–48 h (Whittington *et al*. 2000). Active feeding by large populations of capsalid monogeneans on mucus and epithelial cells of the host fish can cause haemorrhage, inflammation and mucus hyperproduction (Paperna, 1991). Heavily infected fish may stop feeding, and then their body colour darkens, they swim erratically and rub against the net, which may result in dermal ulceration and subsequent bacterial invasion (Woo *et al*. 2002).

To prevent infestation by *N. girellae*, a freshwater dip of 3–5 min is often used to dislodge the parasites from the host fishes (Leong, 1997), but long-term treatments cause damage to cultured fish (Roberts and Powell, 2003*a*, *b*). The treatment requires additional labour and causes stress to the fish (Kim and Choi, 1998). An effective treatment with medicated feed may be more practical than bath treatments. When administered orally, praziquantel is an effective chemotherapeutic compound against skin monogeneans (Okabe, 2000; Hirazawa *et al*. 2004)

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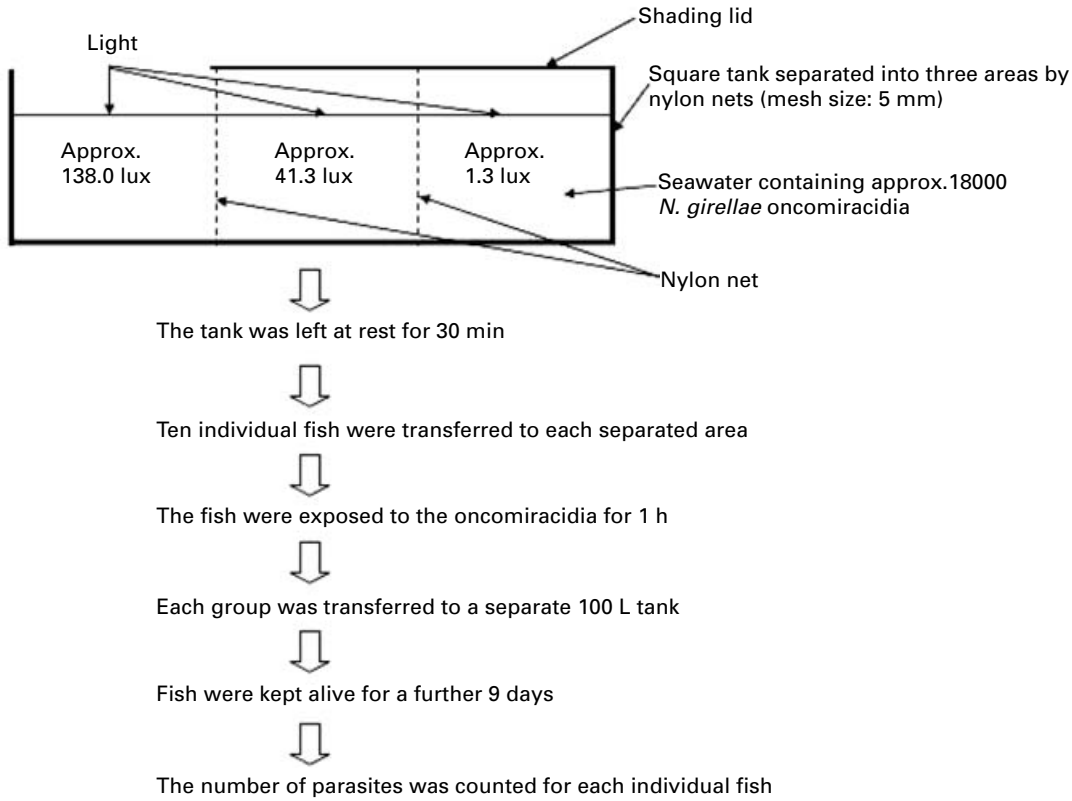

Fig. 1. Outline of the protocol to assess the response of *Neobenedenia girellae* oncomiracidia to brightness.

which feed on epithelium and against gill monogeneans (Hirazawa *et al*. 2000; Kim *et al*. 2001) which feed on blood. The recommended treatment against *N. girellae* on *S. quinqueradiata* is an oral dose of praziquantel at 150 mg per kg body weight per day for 3 successive days (Okabe, 2000). However, the appetite of fish tends to decrease, and fish vomit when fed pellets medicated with praziquantel (Hirazawa *et al*. 2004). In addition, praziquantel administered orally is much harder to deploy to epithelial tissues for uptake by epithelial feeders (Okabe, 2000). Therefore, in addition to chemical treatment, biological control measures with no unfavourable effects on the environment should be incorporated into the control strategy. However, much remains to be studied of the biology of *N. girellae*.

N. girellae has 2 pairs of eye spots, and phototactic responses are known to occur only in oncomiracidia (Yoshinaga *et al*. 2000). However, information on the response of *N. girellae* oncomiracidia to brightness has been limited, and the response of oncomiracidia to black-and-white contrast has not been investigated. Information on the response to black-and-white contrast may be useful to develop an effective control method. The present study investigated whether brightness influences *N. girellae* oncomiracidia infection in *P. olivaceus*, when the fish are exposed to oncomiracidia in the same aquarium with areas of different brightness. The response of oncomiracidia to black-and-white contrast was investigated in challenge trials using host fish mucus and whole live fish.

MATERIALS AND METHODS

Fish rearing

Rearing methods for *V. variegatus* (see Hirazawa *et al*. 1999) and *P. olivaceus* (see Kumai, 2000) were used to establish the flow rates, aeration and feeding rates. Aerated tanks (of 100, 500 or 1000 litre capacity) containing *V. variegatus* or *P. olivaceus* were supplied with seawater previously filtered through sand and irradiated with ultraviolet (UV) light ($\sim$50 000 μw·s/cm^2 for a Flonlizer 4 l unit; Chiyoda Kohan, Ltd, Japan). The mean salinity, pH and chemical oxygen demand of seawater used in our laboratory was $\sim$34 parts per million, 8·1 and 1·0 mg/l, respectively, throughout the year. The fish were fed a commercial extruded 2 mm pellet diet (Nippon Suisan Kaisha Ltd, Japan) twice a day. The daily feeding rate was 3% of body weight.

Uninfected fish

P. olivaceus (500 individuals) weighing approximately 5 g each were obtained from a local juvenile flounder producer in Oita Prefecture. They were maintained in a 1000 litre tank. *V. variegatus* (500 individuals), weighing approximately 8 g each and

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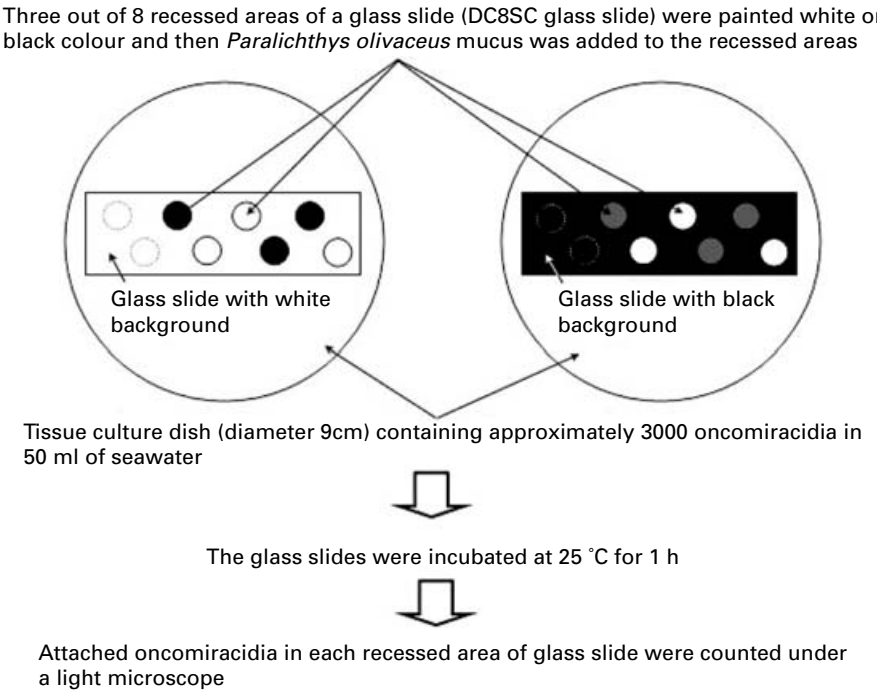

Fig. 2. Outline of the protocol to assess the response of *Neobenedenia girellae* oncomiracidia to black-and-white contrast.

hatched in our laboratory, were maintained in a 500 litre tank. The skin surface of 10 fish of each species, sampled randomly, was examined under a microscope to confirm that the fish were not infected with *N. girellae* or other skin parasites before each experiment. Their gills were also examined using a microscope to confirm that the fish were not infected by gill parasites.

Oncomiracidia of N. girellae

The source of *N. girellae* and its propagation on *V. variegatus* has been described previously (Hirazawa *et al*. 2004; Hatanaka *et al*. 2005). Eggs of *N. girellae* have filaments that can easily entangle with each other, and many such egg masses can further entangle with a net cage (Ogawa and Yokoyama, 1998). We collected the eggs efficiently by putting 5-cm-square nylon nets (mesh size 5 mm) into a 100 litre tank, in which *V. variegatus* infected with the parasites were maintained. Eggs entangled in the nets were incubated in a 300 ml plastic beaker containing filtered seawater (20 °C). The filtered seawater was exchanged every day during incubation. Within 12 h of hatching, oncomiracidia were used for the experiments.

Assessment of response by N. girellae *oncomiracidia to brightness in a challenge trial*

The experiment was conducted twice (Experiments I and II). Thirty uninfected specimens of *P. olivaceus* (mean weight: 38·6 g in Exp. I; mean weight: 47·2 g in Exp. II) were transferred to one tank of 200 litre capacity in each experiment. The fish were acclimatized for 1 week. The tanks were aerated and supplied with sand-filtered and UV-irradiated seawater. The fish were fed, and the temperature was maintained at 25 ± 1 °C (using a heating device) throughout the experiment. A tank (inside dimension: length 96·5 × width 30·0 × height 20·0 cm) was divided into 3 areas with nylon nets (mesh size was 5 mm) (Fig. 1). Then 32 litres of seawater at 25 °C containing ~18 000 *N. girellae* oncomiracidia were put into the tank. The tank was aerated for 3 min. The aeration supply to the tank was stopped, the tank was covered with a shading lid (length 33 × width 33 × height 0·5 cm; vinyl chloride), and then the tank was rested for 30 min. The brightness of each of the 3 areas in the tank was ~1·3 lux, 41·3 lux and 138·0 lux, respectively, measured using a lux meter (Fine lux meter FLX-1330, Tokyo Asahi Kikai Ltd, Japan); 3 locations in each area were measured and the values were averaged. The acclimatized fish were divided into 3 groups: low brightness group (1·3 lux), middle brightness group (41·3 lux), high brightness group (138·0 lux). The shading lid of the square tank was opened, each group was transferred to each area and the tank was covered again. The fish were exposed to the oncomiracidia for 1 h. After the exposure to oncomiracidia, individual groups of fish were transferred to separate 100 litre tanks of the same brightness (~160 lux). The fish were kept alive for a further 9 days after infection. At the end of

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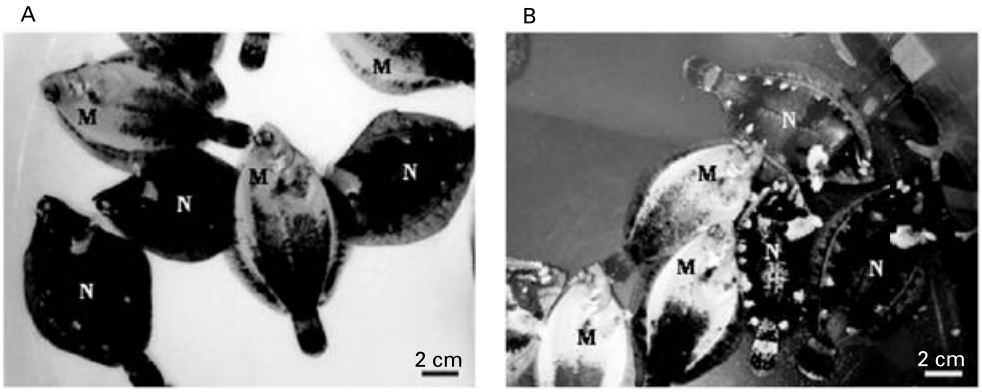

Fig. 3. *Verasper variegatus*. (A) White background. (B) Black background. Dark-coloured *V. variegatus* on their uppermost ocular side are normal fish (N). Light-coloured *V. variegatus* on their uppermost ocular side are mal-coloured fish (M).

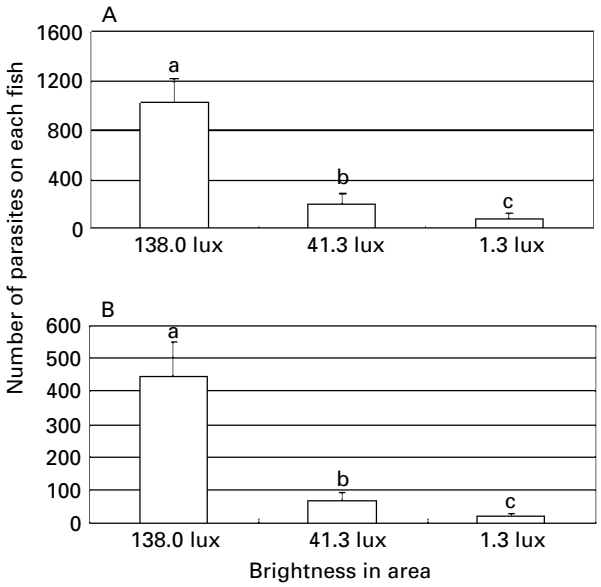

Fig. 4. Infection levels of *Neobenedenia girellae* on *Paralichthys olivaceus* exposed to oncomiracidia in the same aquarium that had areas with different brightness. (A) Experiment I. (B) Experiment II. Values are means and standard deviations. The values with different superscripts are significantly different at $P<0{\cdot}05$.

the experiment, each fish was dipped separately in fresh water for 10 min to dislodge the parasites from the host (Leong, 1997). The total number of dislodged *N. girellae* per fish was counted under a stereomicroscope. Analysis of variance (one-way ANOVA) followed by Fishers PLSD *post hoc* test was used to identify the statistical difference in the numbers of parasites between the groups. Significance was accepted at $P<0{\cdot}05$. The data were analysed using the Statcel statistical software package (OMS, Inc. Japan).

Assessment of response by N. girellae *oncomiracidia to black-and-white contrast in host fish mucus trials*

A DC8SC glass slide (Neuro Probe, Inc., USA) has 8 recessed circular areas of 6·4 mm in diameter and 32 mm² in area. The slide outside the recessed areas and 5 recessed areas were painted white or black using permanent markers and the remaining 3 recessed areas were painted the opposite colour (Fig. 2). Skin mucus (50 μl) from *P. olivaceus* was added to each of 6 (3 areas of each colour) of the 8 recessed areas. The glass slide was incubated for 3 h at 30 °C to dry the mucus to prevent from sloughing off the slide in seawater. The glass slide was put into a tissue culture dish (diameter 9 cm), containing ~3000 oncomiracidia within 12 h of hatching in 50 ml of seawater, and was incubated at 25 °C for 1 h. Then, the glass slide was removed from the dish and the attached mucus-treated oncomiracidia in each recessed area were counted under a light microscope. The oncomiracidia counted shed their ciliated epidermal cells and attached to the mucus with the haptor unfolded. The experiment was conducted 3 times (Exps I, II and III) and mucus from different fish and different batches of parasites were used in Exps I, II and III. The data were tested using a *t*-test to find significant differences between the white and black groups. Significance was accepted at $P<0{\cdot}05$. All analyses were conducted using the Statcel statistical software package.

Assessment of response by N. girellae *oncomiracidia to black-and-white contrast in challenge trials*

Uninfected *V. variegatus* (mean weight: 18·8 g) were randomly divided into 2 groups, each group consisting of 10 'normal' (colour of their uppermost ocular side was *dark*) and 10 'mal-coloured' (colour of their uppermost ocular side was *light*) fish (Fig. 3). Mal-coloured fish (pigment abnormalities) in hatchery-reared juveniles of flatfish are often observed (Venizelos and Benetti, 1999). These abnormal fish suffer from thyroid hormone deficiency (Okada *et al.* 2005) and dietary deficiencies (Kanazawa, 1993). One group was kept in a 100 litre tank with a white bottom (Fig. 3A), and the other

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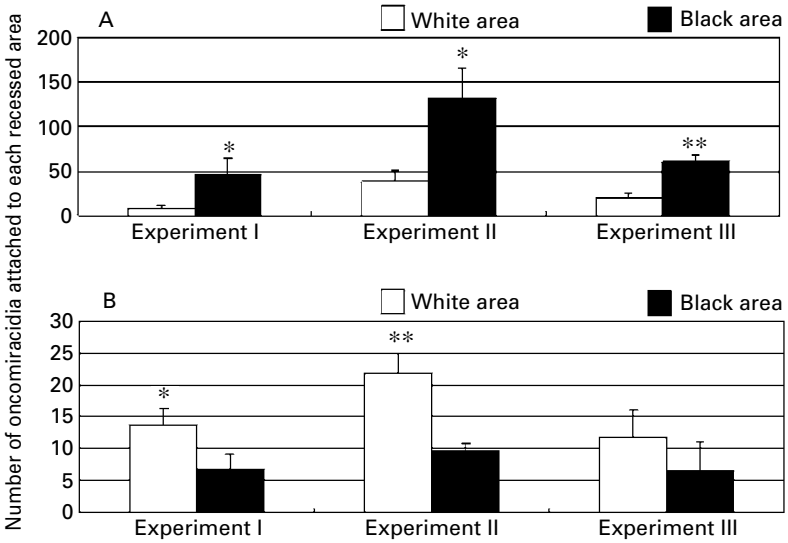

Fig. 5. Response of *Neobenedenia girellae* oncomiracidia to mucus from *Paralichthys olivaceus* on black and white areas with a white or black background. (A) White background. (B) Black background. Values are means and standard deviations. Asterisks indicate a significant difference (*$P<0{\cdot}05$, **$P<0{\cdot}01$).

group was kept in a 100 litre tank with a black bottom (Fig. 3B). Fish were acclimatized for a week. The tanks were aerated and supplied with seawater. The fish were fed the diet, and the temperature was maintained at $25\pm1\,^{\circ}$C throughout the experiment. After acclimatization, ~3000 oncomiracidia were put into each tank within 12 h of hatching. The seawater supply was discontinued for 1 h to assist in establishing the infection, and the aeration supply to the tank was stopped for 1 h. The experiment continued for 9 days after the exposure to oncomiracidia. At the end of the experiment, each fish was dipped in fresh water for 10 min to dislodge the parasites from the host and the total number of dislodged *N. girellae* per fish was counted. The data were tested using the *t*-test to find significant differences between normal and mal-coloured fish in each group. Significant differences were accepted at $P<0{\cdot}05$. All analyses were made using the Statcel statistical software package.

RESULTS

Assessment of response by N. girellae *oncomiracidia to brightness in a challenge trial*

We investigated whether brightness influences the infection by *N. girellae* oncomiracidia when *P. olivaceus* were exposed to larvae in the same aquarium with areas of different brightness. The number of parasites on fish reared in 138 lux, the brightest condition, was significantly higher than on fish of the other 2 test groups, indicating that the infection was influenced by brightness (Fig. 4).

Assessment of response by N. girellae *oncomiracidia to black-and-white contrast in host fish mucus trials*

The response of *N. girellae* oncomiracidia to *P. olivaceus* mucus added to a glass slide that had black-and-white contrast (see Fig. 2) was examined. Glass slides with a white background had markedly more *N. girellae* oncomiracidia attached to the black areas compared with white areas (Fig. 5A), and the number of oncomiracidia attached to black areas was significantly higher than for white areas in Exps I–III.

Glass slides with a black background had markedly more *N. girellae* oncomiracidia attached to the white areas compared with the black areas (Fig. 5B), and the number of oncomiracidia attached to white areas was significantly higher than for black areas in Exps I and II, although not significantly higher in Exp. III.

Assessment of response by N. girellae *oncomiracidia to black-and-white contrast in challenge trials*

The response of *N. girellae* oncomiracidia to dark-coloured *V. variegatus versus* light-coloured *V. variegatus* in a tank with a white or a black bottom was examined. In a tank with a white bottom, the number of parasites on dark-coloured fish was significantly larger than for light-coloured fish (Fig. 6A). Conversely, the number of parasites on mal-coloured light-coloured fish in a tank with a black bottom tended to be higher compared with normal dark-coloured fish (Fig. 6B), but the difference was not statistically significant ($P=0{\cdot}265$).

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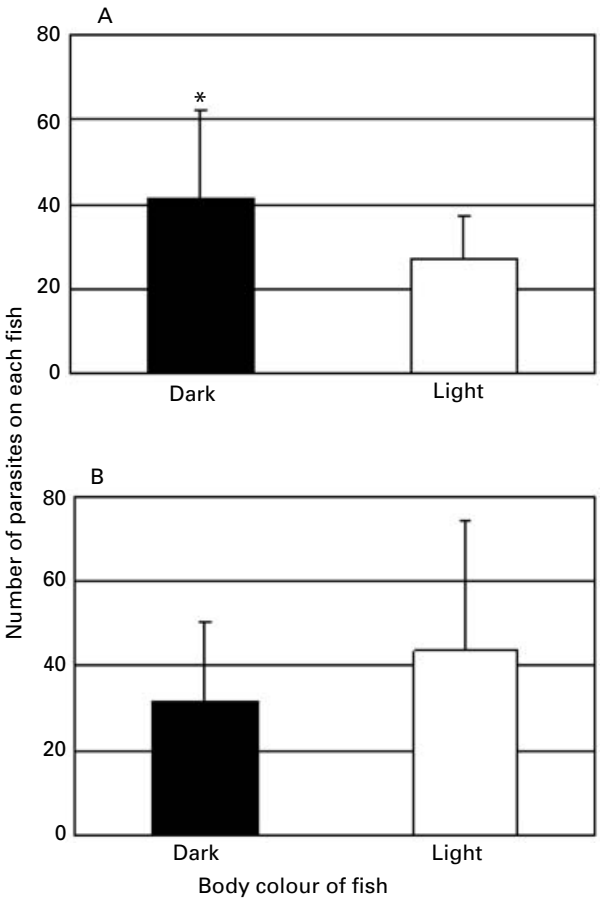

Fig. 6. Response of *Neobenedenia girellae* oncomiracidia to dark-coloured *Verasper variegatus* and light-coloured *Verasper variegatus* with white or black background. (A) White background. (B) Black background. Values are means and standard deviations. Asterisk indicates a significant difference from the values between dark-coloured fish and light-coloured fish ($*P<0{\cdot}05$). In a tank with black bottom (B), the number of parasites on light-coloured fish tended to be high compared with dark-coloured fish, but the difference between the 2 different coloured fishes was not statistically significant ($P=0{\cdot}265$).

DISCUSSION

Many monogenean oncomiracidia have eyes, and their orientation with respect to light is likely to be important in host-finding, if the eyes have a phototactic function. There have been few studies of the light-orientated behaviour of monogenean larvae. Photo-positive behaviour has been shown for the oncomiracidia of *Diplozoon paradoxum* (see Bovet, 1967) and *Discocotyle sagittata* (see Paling, 1969). On the other hand, Fournier (1976) referred to photo-negative behaviour in the oncomiracidium of *Euzetrema knoepfferi*. Oncomiracidia of dactylogyrids have 2 phases of phototaxis (Bychowsky, 1957); during the first phase, the larvae are positively phototactic and the haptor is folded in such a way that the hooks do not protrude; in the second phase, the larvae are photo-negative with an unfolded haptor and protruding hooks. There is also evidence that larvae of other monogeneans, such as *Discocotyle sagittata* (see Paling, 1969) and *Entobdella soleae* (see Kearn, 1980), display age-dependent photoresponses. *N. girellae* has 2 pairs of eye spots, and their phototactic role is known only for the oncomiracidia which are positively phototactic (Yoshinaga *et al.* 2000). The current study provided evidence that brightness influences the infection of fish with *N. girellae* oncomiracidia, and showed that fish tend to harbour more *N. girellae* under brighter conditions. However, further studies are needed to assess the responses of *N. girellae* oncomiracidia to brightness according to their age. In this study, *N. girellae* oncomiracidia (within 12 h of hatching) were used to obtain sufficient numbers for use in the experiments. These groups comprised larvae of various ages between 0 and 12 h old, and these oncomiracidia may differ in their photoresponses.

In most cases in Japan, marine fish are cultured in floating off-shore net cages. The positive phototactic behaviour of *N. girellae* oncomiracidia may be used to prevent outbreaks in aquaculture systems. For instance, *Seriola* species are in some cases cultured in sunken cage systems, because floating off-shore net cages collapse occasionally during typhoons which hit Japan in summer. The sunken cage system may reduce the chances of fish being exposed to *N. girellae* oncomiracidia. In general, light intensity at the water surface is ~15 000 lux during the day (Sakakura and Tsukamoto, 1997) and attenuates according to depth. The light intensity at a depth of 29·5 m on the Pacific coast is one hundredth of that at the water surface (Hirano, 1998).

Successful host-finding and attachment are crucial for parasite survival. Some studies have shown that monogenean eggs and oncomiracidia respond to environmental stimuli (i.e. light, gravity, mechanical disturbance, water currents, chemical substances and mucus pH), indicating that such responses increase the probability of successful host-finding (Ktari, 1969; Paling, 1969; Kearn, 1974, 1980; Whittington and Kearn, 1986, 1989; Yoshinaga *et al.* 2000; Buchmann and Lindenstrøm, 2002; Hirazawa *et al.* 2003). The response by *N. girellae* oncomiracidia to black-and-white contrast detected in the present study showed that more *N. girellae* oncomiracidia attached to black areas and dark-coloured fish in a white background, and, conversely, more *N. girellae* oncomiracidia tended to attach to white areas and light-coloured fish on a black background. These results show that black-and-white contrast may be important in host-finding by *N. girellae* oncomiracidia, which react more strongly to black objects on a white background than white objects on a black background. These findings may be applied to aquaculture to prevent or reduce *N. girellae* infection. In the case of *P. olivaceus*, which is mostly farmed in land-based tanks with running seawater,

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a rearing tank with a similar colour to the fish, in order to reduce black-and-white contrast, may suppress the chances of the fish encountering *N. girellae*. Usually, flatfish are able to change their pigmentation, and become camouflaged to resemble their surroundings. The camouflage is useful in deceiving enemies and prey, and may also suppress the chances of the fish encountering *N. girellae*. However, most fish species darken their bodies when infected with pathogens (Woo *et al*. 2002). This is the case in *N. girellae* infection, which often relates to relatively large numbers of worms (Hirazawa, unpublished observation).

N. girellae has been introduced into Japan through imported, wild *S. dumerili* fingerlings (Bondad-Reantaso *et al*. 1995) and is now recognized on some other cultured marine fishes. *N. girellae* eggs are able to hatch at 18–30 °C but they do not hatch at 15 °C (Bondad-Reantaso *et al*. 1995). These observations on hatching show that this parasite has adapted to high water temperature. Infection by *N. girellae* among fishes in southwest Japan substantially decrease in the winter months but the infection persists even in those months in subtropical areas, such as the Okinawa and Ogasawara Islands (Bondad-Reantaso *et al*. 1995). *S. dumerili* is distributed in these subtropical areas but *P. olivaceus* and *V. variegatus* are not. These observations suggest that *N. girellae* is not a natural parasite of *P. olivaceus* and *V. variegatus*. The susceptibility of *V. variegatus* to *N. girellae* is low compared with *P. olivaceus* (see Hirazawa *et al*. 2004) but the growth of the parasite on *V. variegatus* is similar to that on *P. olivaceus*. Differences in susceptibility among other fish species to *N. girellae* infection have not been investigated. The host species differences in susceptibility to this parasite may affect the behaviour of *N. girellae* oncomiracidia in relation to the black-and-white contrast. In this study, *P. olivaceus* mucus and live *V. variegatus* were used for challenge trials because the aim of this study was to assess *N. girellae* oncomiracidia behaviour to the black-and-white contrast.

The ocellus in many free-living and parasitic platyhelminths consists of a pigment cell, rhabdomeres and microvillus-like out-growths of dendrites connected to a sensory cell outside of the receptor (Kearn and Baker, 1973; Fournier and Combes, 1978; Kearn, 1978). These structures may change the position of the receptors relative to the adjacent tissues and the incoming light and, thus, may modify phototactic reaction (Rohde and Watson, 1991). Photoreceptors may be responsible for directed swimming toward the light, i.e. they may mediate phototactic responses that lead the larva into a habitat in which infection is possible (Rohde and Watson, 1991).

In this study, brightness influenced the *N. girellae* oncomiracidial infection in fish, the brighter conditions resulting in fish with greater numbers of *N. girellae*. The response by *N. girellae* oncomiracidia to black-and-white contrast suggests that black-and-white contrast may be important for host-finding by *N. girellae* oncomiracidia. Further studies are needed to assess the responses of *N. girellae* oncomiracidia of different ages to brightness and black-and-white contrast. Similarly, to determine whether these features may be used in different aquaculture systems, such as sunken cages or fish-skin-coloured aquaria to prevent infection. The apparent simplicity of the positive phototactic behaviour and the response to black-and-white contrast may lead to the development of practical and inexpensive approaches for the control of *N. girellae* outbreaks in fish farms.

REFERENCES

Bondad-Reantaso, M. G., Ogawa, K., Fukudome, M. and Wakabayashi, H. (1995). Reproduction and growth of *Neobenedenia girellae* (Monogenea: Capsalidae), a skin parasite of cultured marine fishes of Japan. *Fish Pathology* **30**, 227–231.

Bovet, J. (1967). Contribution à la morphologie et à la biologie de *Diplozoon paradoxum* V. Nordmann, 1832. *Bulletin de la Société Neuchâteloise des Sciences naturelles* **90**, 64–159.

Buchmann, K. and Lindenstrøm, T. (2002). Interactions between monogenean parasites and their fish host. *International Journal for Parasitology* **32**, 309–319.

Bychowsky, B. E. (1957). *Monogenetic Trematodes, their Classification and Phylogeny*. Academy of Science, USSR, Moscow and Leningrad. (In Russian.)

Fournier, A. (1976). Evolution des ultrastructures d'un monogène au cours du cycle. *Bulletin de la Société zoologique de France* **101**, 1059–1060.

Fournier, A. and Combes, C. (1978). Structure of photoreceptors of *Polystoma integerrimum* (Platyhelminthes, Monogenea). *Zoomorphology* **91**, 147–155.

Hatanaka, A., Umeda, N., Yamashita, S. and Hirazawa, N. (2005). A small ciliary surface glycoprotein of the monogenean parasite *Neobenedenia girellae* acts as an agglutination/immobilization antigen and induces an immune response in the Japanese flounder *Paralichthys olivaceus*. *Parasitology* **131**, 591–600.

Hirano, T. (1998). *Marine Coastal Environment*. 1st Edn. Fuji Technosystem, Tokyo. (In Japanese.)

Hirazawa, N., Hara, T., Mitsuboshi, T, Okazaki, J. and Hata, K. (1999). Iodophor disinfection of eggs of spotted halibut *Verasper variegatus* and red sea bream *Pagrus major*. *Fisheries Science* **65**, 333–338.

Hirazawa, N., Mitsuboshi, T., Hirata, T. and Shirasu, K. (2004). Susceptibility of spotted halibut *Verasper variegatus* (Pleuronectidae) to infection by the monogenean *Neobenedenia girellae* (Capsalidae) and oral therapy trials using praziquantel. *Aquaculture* **238**, 83–95.

Hirazawa, N., Ohtaka, T. and Hata, K. (2000). Challenge trials on the anthelmintic effect of drugs and natural agents against the monogenean *Heterobothrium*

okamotoi in the tiger puffer *Takifugu rubripes*. *Aquaculture* **188**, 1–13.

Hirazawa, N., Oshima, S., Mitsuboshi, T. and Yamashita, S. (2003). Mucus pH of the tiger puffer *Takifugu rubripes* is an important factor for host identification by the monogenean *Heterobothrium okamotoi*. *Parasitology* **127**, 225–230.

Kanazawa, A. (1993). Nutritional mechanisms involved in the occurrence of abnormal pigmentation in hatchery-reared flatfish. *Journal of the World Aquaculture Society* **24**, 162–166.

Kearn, G. C. (1974). The effect of fish skin mucus on hatching in the monogenean parasite *Entobdella soleae* from the skin of the common sole, *Solea solea*. *Parasitology* **68**, 173–188.

Kearn, G. C. (1978). Eyes with, and without, pigment shields in the oncomiracidium of the monogenean parasite *Diplozoon paradoxum*. *Zeitschrift für Parasitenkunde* **53**, 35–47.

Kearn, G. C. (1980). Light and gravity responses of the oncomiracidium of *Entobdella soleae* and their role in host location. *Parasitology* **81**, 71–91.

Kearn, G. C. and Baker, N. O. (1973). Ultrastructural and histochemical observations on the pigmented eyes of the oncomiracidium of *Entobdella soleae*, a monogenean skin parasite of the common sole, *Solea solea*. *Zeitschrift für Parasitenkunde* **41**, 239–254.

Kim, K. H. and Choi, E. S. (1998). Treatment of *Microcotyle sebastis* (Monogenea) on the gills of cultured rockfish (*Sebastes schelegeli*) with oral administration of mebendasole and bithinol. *Aquaculture* **167**, 115–121.

Kim, K. H., Lee, E. H., Kwon, S. R. and Cho, J. B. (2001). Treatment of *Microcotyle sebastis* infestation in cultured rockfish *Sebastes schlegeli* by oral administration of praziquantel in combination with cimetidine. *Diseases of Aquatic Organisms* **44**, 133–136.

Ktari, M. H. (1969). Recherches sur l'anatomie et la biologie de *Microcotyle salpae* Parona et Perugia. 1890 prasite de *Box salpa* L. (Téléostéen). *Annales de Parasitologie Humaine et Comparée* **44**, 425–440.

Kumai, H. (2000). *Aquaculture of Marine Fishes*. Sobunsha, Tokyo. (In Japanese.)

Leong, T. S. (1997). Control of parasites in cultured marine finfishes in Southeast Asia-an overview. *International Journal for Parasitology* **27**, 1177–1184.

Ogawa, K., Bondad-Reantaso, M. G., Fukudome, M. and Wakabayashi, H. (1995). *Neobenedenia girellae* (Hargis, 1955) Yamaguti, 1963 (Monogenea: Capsalidae) from cultured marine fishes of Japan. *Journal of Parasitology* **81**, 223–227.

Ogawa, K. and Yokoyama, H. (1998). Parasitic diseases of cultured marine fish in Japan. *Fish Pathology* **33**, 303–309.

Okabe, K. (2000). Chemotherapeutic drug (Hada-clean) of oral administrating type to control fish parasites. *Doyaku Kenkyu* **60**, 1–12. (In Japanese.)

Okada, N., Morita, T. and Tagawa, M. (2005). Thyroid hormone deficiency in abnormal larvae of the Japanese flounder *Paralichthys olivaceus*. *Fisheries Science* **71**, 107–114.

Paling, J. E. (1969). The manner of infection of trout gills by the monogenean parasite *Discocotyle sagittata*. *Journal of Zoology* **159**, 293–309.

Paperna, I. (1991). Diseases caused by parasites in the aquaculture of warm water fish. *Annual Review of Fish Diseases* **1**, 155–194.

Roberts, S. D. and Powell, M. D. (2003*a*). Comparative ionic flux and gill mucous cell histochemistry: effect of salinity and disease status in Atlantic salmon (*Salmo salar* L.). *Comparative Biochemistry and Physiology* **134**(A), 525–537.

Roberts, S. D. and Powell, M. D. (2003*b*). Reduced total hardness of fresh water enhances the efficacy of bathing as a treatment for amoebic gill disease in Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases* **26**, 591–599.

Rohde, K. and Watson, N. A. (1991). Ultrastructure of pigmented photoreceptors of larval *Multicotyle purvisi* (Trematoda, Aspidogastrea). *Parasitology Research* **77**, 485–490.

Sakakura, Y. and Tsukamoto, K. (1997). Effects of seawater temperature and light intensity on aggressive behavior in the juvenile yellowtail. *Fisheries Science* **63**, 42–45.

Venizelos, A. and Benetti, D. D. (1999). Pigment abnormalities of the flatfish. *Aquaculture* **176**, 181–188.

Whittington, I. D., Chisholm, L. A. and Rohde, K. (2000). The larvae of Monogenea (Platyhelminthes). *Advances in Parasitology* **44**, 139–232.

Whittington, I. D. and Kearn, G. C. (1986). Rhythmical hatching and oncomiracidial behavior in the hexabothriid monogenean *Rajonchocotyle emarginata* from the gills of *Raja* spp. *Journal of the Marine Biological Association of the United Kingdom* **66**, 93–111.

Whittington, I. D. and Kearn, G. C. (1989). Rapid hatching induced by light intensity reduction in the polypisthocotylen monogenean *Plectanocotyle gurnardi* from the gills of *Gurnards triglidae* with observations on the anatomy and behaviour of the oncomiracidium. *Journal of the Marine Biological Association of the United Kingdom* **69**, 609–624.

Woo, P. T. K., Bruno, D. W. and Lim, L. H. S. (2002). *Diseases and Disorders of Finfish in Cage Culture*. CABI Publishing, London.

Yoshinaga, T., Nagakura, T., Ogawa, K. and Wakabayashi, H. (2000). Attachment-inducing capacities of fish tissue extracts on oncomiracidia of *Neobenedenia girellae* (Monogenea, Capsalidae). *Journal of Parasitology* **86**, 214–219.