Lipid content and fatty acid composition of the monogenean *Neobenedenia girellae* and comparison between the parasite and host fish species

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SUMMARY

Neobenedenia girellae, a capsalid monogenean, is a destructive fish parasite. We studied the lipid content and fatty acid composition of *N. girellae* and the skin and cutaneous mucus of a host fish, the amberjack *Seriola dumerili* (Carangidae). The lipid content of adult *N. girellae* was less than one-fourth that of both the skin and cutaneous mucus of its host. Adult *N. girellae*, *S. dumerili* skin and mucus had a relatively high weight-percentage of C16 : 0, C18 : 1(n-9), C18 : 0 and C22 : 6(n-3) fatty acids. When *S. dumerili* were fed a diet supplemented with [^{13}C] fatty acids, [^{13}C] fatty acids were detected in *S. dumerili* skin and adult *N. girellae* on *S. dumerili*, but no [^{13}C] fatty acids were detected in the *S. dumerili* cutaneous mucus. In addition, the epidermis of *S. dumerili*, attached with *N. girellae*, was markedly thin. These results suggest that *N. girellae* feeds primarily on host epithelial cells. We then infected 2 host fishes, *S. dumerili* and the spotted halibut *Verasper variegatus* (Pleuronectidae; a host less susceptible to *N. girellae* infection), and compared the fatty acid composition of *N. girellae* with that of the skin and cutaneous mucus of the hosts. The fatty acid profiles from all samples were qualitatively and quantitatively similar. Thus, the fatty acid composition of the host may not contribute to the difference in susceptibility between *S. dumerili* and *V. variegatus*. These results may serve to develop new strategies for the control of *N. girellae* infections.

Key words: Monogenea, *Neobenedenia girellae*, lipid content, fatty acid composition, *Seriola dumerili*, *Verasper variegatus*.

INTRODUCTION

Many parasites are serious pathogens in intensive aquaculture in Japan (Ogawa and Yokoyama, 1998). One such parasite, *Neobenedenia girellae*, a capsalid monogenean, is problematic because it can cause high mortality in host fishes (Ogawa *et al*. 1995) and it has broad host specificity (Bondad-Reantaso *et al*. 1995). Some commercially important cultured fishes, such as the amberjack *Seriola dumerili* and the yellowtail *S. quinqueradiata* (Carangidae), the Japanese flounder *Paralichthys olivaceus* (Paralichthyidae) and the spotted halibut *Verasper variegatus* (Pleuronectidae), become infected with this monogenean (Ogawa and Yokoyama, 1998; Hirazawa *et al*. 2004). However, among these host fish species, *S. dumerili* is most susceptible to *N. girellae* (Hirazawa *et al*. 2004; Ohno *et al*. 2008).

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. 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, their body colour darkens, and they swim erratically and rub against the net, which may result in dermal ulceration and subsequent bacterial invasion (Woo *et al*. 2002).

Lipids play a vital role in long-term survival and in the completion of the life cycle of endoparasitic parasites (Furlong, 1991; Sherman, 1998). Parasitic protozoa require an exogenous source of essential lipids for growth, differentiation and life-cycle completion (Soudant and Chu, 2001). Utilization of fatty acids in serum is thought to be necessary for survival of the protozoan *Plasmodium falciparum* in erythrocytes. Characterization of the parasite fatty acid metabolism was important for developing a new strategy for controlling malaria (Mi-ichi *et al*. 2006). However, lipid content and fatty acid composition of *N. girellae* have not been investigated. Information regarding the lipid content and fatty

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acid composition of *N. girellae* would help to control this parasite.

In this study, the lipid content and fatty acid composition of adult *N. girellae* was investigated. We also investigated whether the fatty acids of the parasite are derived from host fish. In addition, the fatty acid composition of cutaneous mucus and skin of 2 host fish species: *S. dumerili* and *V. variegates* was analysed, the former being more susceptible to the parasite. Furthermore, histological sections of host skin infected with *N. girellae* were examined.

MATERIALS AND METHODS

Fish rearing

The rearing methods of Kumai (2000) for *S. dumerili* and Hirazawa *et al.* (1999) for *V. variegatus* were used to establish aeration and feeding rates. Aerated tanks (200 l, 1000 l or 2000 l capacity) containing *S. dumerili* or *V. variegatus* were supplied with seawater (~1·2 l/min per 100 l tank volume) previously filtered through sand and irradiated with ultraviolet light (~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 were ~34 parts per million, 8·1 and 1·0 mg/l, respectively, throughout the year. The fish were fed a commercial 1–3 mm extruded pellet diet (Nippon Suisan Kaisha Ltd, Japan) twice a day depending on fish size. For uninfected fish, the daily feeding rate was 3% of body weight. Experimental fish were fed pellet diets once a day at a rate of 2% of body weight to ensure that fish were completely consuming the experimental diet. The experimental diet (basal diet) was composed of brown meal (40%), squid meal (30·0%), krill meal (10·0%), wheat gluten (3·0%), guar gum (2·0%), sardine oil (6·0%), soybean lecithin (2·0%), vitamin mixture (4·0%) and mineral mixture (3·0%).

Uninfected fish

Seriola dumerili (500 individuals) weighing ~2 g each and caught by local fisherman, were transferred to our laboratory. Ten fish were determined to be free from infection with *N. girellae* by examination, and then were treated with a freshwater bath for 3 min. A freshwater dip of 3–5 min is often practiced to dislodge parasites from the host (Leong, 1997). The fish were then treated with a bath of sodium nifurstyrenate, a synthetic antibacterial chemical, at a concentration of 10 ppm for 1 h to prevent bacterial diseases due to handling during the freshwater bath treatment. The fish were maintained in a 1000-l tank. *Verasper variegatus* (500 individuals), weighing ~8 g each and hatched in our laboratory, were maintained in a 2000 l 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 because the rearing period was more than 3 months. Their gills were also examined using a microscope to confirm that the fish were not infected by gill parasites. The sampled fish were killed by removal of their abdominal vertebrae with scissors just before examination.

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 entangle with each other and with nylon nets suspended in the aquarium (Kearn *et al.* 1992). Eggs were collected efficiently by putting 5 cm square nylon nets (mesh size 5 mm) into a 100 l 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 changed every day during incubation. Within 12 h of hatching, oncomiracidia were used for the experiments. The seawater containing the hatched oncomiracidia in the 300 ml plastic beaker was agitated and 1 ml of the seawater was drawn 5 times. The total number of hatched oncomiracidia was estimated as the average number of larvae in the five 1 ml seawater samples.

N. girellae *infection and growth of* S. dumerili *to determine origin of* N. girellae *fatty acids using* ^{13}C (*Experiment 1*)

Adult *N. girellae*, *S. dumerili* cutaneous mucus and skin were prepared to analyse their lipid content and fatty acid composition, and to determine the origin of the parasite fatty acids. Twenty uninfected *S. dumerili* specimens, weighing ~70 g each, were randomly divided into 2 groups and each group was transferred to a 200 l tank. The fish were acclimatized for 1 week and the fish were fed a basal diet throughout the acclimatization period. Each tank was aerated and supplied with seawater and the temperature was maintained at 25 ± 1 °C using a heating device (WTCA-602L heating-cooling unit, Earth Co. Ltd, Tokyo, Japan) throughout the trial. After acclimatization, ~1600 hatched oncomiracidia (body length: approximately 200 μm) were put into each tank while the seawater supply was discontinued for 1 h to assist in establishing the infection. After the exposure to oncomiracidia, one group was fed the basal diet without [^{13}C] fatty acids for 10 days to investigate the lipid content and the fatty acid composition of adult *N. girellae* and the host *S. dumerili*. The other group was fed the experimental diet supplemented with [^{13}C] fatty acids (Spectra Gases Inc., Japan) (Table 1): 7·1 g [^{13}C] fatty acids/kg basal

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Table 1. Relative proportions of [^{13}C] fatty acids used to supplement the basal diet

[^{13}C] fatty acids	% of total [^{13}C] fatty acids
C14 : 0	1·0
C14 : 1(n-5)	1·0
C15 : 0	1·0
C16 : 0	22·0
C16 : 1(n-7)	3·5
C16 : 2(n-6)	8·5
C16 : 3(n-3)	9·5
C17 : 0	1·0
C18 : 0	2·5
C18 : 1(n-9)	8·5
C18 : 1(n-7)	1·0
C18 : 2(n-6)	26·5
C18 : 3(n-3)	14·5
C20 : 0	1·0
C22 : 0	1·0

diet (140 mg [^{13}C] fatty acids/kg body weight/day) for 10 days to determine the origin of the parasite fatty acids. [^{12}C] is the major stable carbon-12 isotope and makes up for 98·98% of all of natural carbon derived from the diet. On the other hand, [^{13}C] is the minor stable carbon-13 isotope. It makes up about 1·1% of all natural carbon. Correspondingly, the major stable isotope of carbon indicates [^{12}C]. To generate the experimental diet supplemented with [^{13}C] fatty acids, [^{13}C] fatty acids were mixed into the paste of the basal diet, and a 2·5 mm diameter pellet was formed by passing the paste through a disc pelleter (model F-5, Fuji Paudal, Japan). The diet pellets were stored at −20 °C. The experiment continued for 11 days after the exposure to oncomiracidia; the fish were reared on each experimental diet for 10 days and were sampled on day 11. At the end of the experiment, cutaneous mucus was collected by keeping each fish in a plastic bag for 10 min without water. The mucus from each experimental group was transferred from the bag to a 50 ml centrifuge tube. The mucus solution was centrifuged at 1600 ***g*** for 15 min at 4 °C, and the supernatant was collected and stored at −80 °C until analysed for fatty acid composition. Each fish was then dipped in freshwater for 10 min to dislodge the parasites from the host and the total number of dislodged *N. girellae* per fish was counted. The body length, including haptor, of 50 parasites sampled from each treatment group was measured using a calibrated ocular micrometer. The dislodged adult *N. girellae* from each experimental group were collected using a cell strainer (Decton Dickinson Labware, USA), 2·7 cm in diameter and 70 μm in mesh opening, and the parasites were trapped when freshwater containing the parasites was passed through the cell strainer. The collected parasites were washed with distilled water, transferred to a 50 ml centrifuge tube and stored at −80 °C until analysed for fatty acid composition. To determine the fatty acid composition of the skin, the fish were wiped off, filleted and peeled. The collected skins from each experimental group were transferred to a 50 ml centrifuge tube and stored at −80 °C.

Comparison of N. girellae *infections of* S. dumerili *and* V. variegatus (*Experiment 2*)

To analyse their lipid content and fatty acid composition, 5 individuals of each fish species, weighing an average of 281·8 g for *S. dumerili*, and 263·9 g for *V. variegatus* were transferred to separate 200 l tanks of aerated seawater. The fish were acclimatized for 1 week. The fish were fed a basal diet and the temperature was maintained at 25 ± 1 °C using a heating device throughout the trial. After acclimatization, ~2000 hatched oncomiracidia (body length approximately 200 μm) were put into each tank while the seawater supply was discontinued for 1 h to assist establishment of the infection. The experiment continued for 11 days after the exposure to oncomiracidia. At the end of the experiment, adult *N. girellae* were counted and measured and cutaneous mucus and skin of each host fish species were collected as described above. The total length of all the fish was measured to calculate the fish surface area. The number of parasites per square centimeter (cm^2) was calculated using the following equations:

the total fish surface area of *S. dumerili* (cm^2) $= 0{\cdot}158 \times (\text{total length})^{2{\cdot}089} \times 2$ (Ohno *et al.* 2008), and

the total fish surface area of *V. variegatus* (cm^2) $= 0{\cdot}359 \times (\text{total length})^{1{\cdot}995} \times 2$ (Kimoto and Sato, 2002).

The data of the number of parasites/cm^2 and *N. girellae* body length were analysed using a *t*-test to find significant differences between *S. dumerili* and *V. variegatus* groups.

Gas chromatography analysis of fatty acids

Host fish skin and cutaneous mucus collected from *N. girellae* were treated by ultrasound and then extracted with MeOH-$CHCl_3$ (1 : 1, v / v). The extracts from each source were filtered and concentrated *in vacuo*. Each lipid concentrate (1·0 mg/extract) was transesterified as follows. Firstly, 1·0 ml of sodium methoxide methanol solution was added to each concentrate and incubated at 80 °C for 10 min. Subsequently, 1·0 ml of boron trifluoride was added to each reaction solution, followed by incubation at 80 °C for 10 min. The resulting suspensions were partitioned between 1·0 ml of iso-octane (2, 2, 4 trimethyl pentane) and 5·0 ml of saturated brine. The fatty acid methyl esters (FAMEs) partitioned in the

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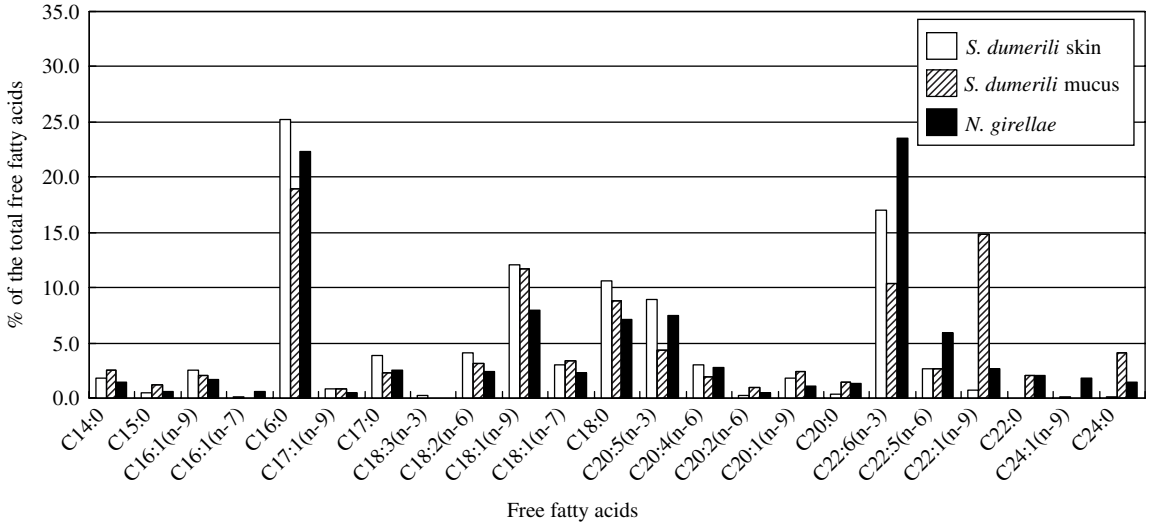

Fig. 1. Fatty acid composition of adult *Neobenedenia girellae* and the skin and cutaneous mucus of the most susceptible host, the amberjack *Seriola dumerili*.

isooctane phase, and 1·0 μl of each sample was automatically injected into a GC-2010 gas chromatograph (Shimadzu Corporation, Japan) equipped with a DB-1HT capillary column (0·250 mm × 15 m df = 0·1 μm, Agilent Technologies, Japan) and then analysed by GCMS-QP2010 mass spectrometer (Shimadzu Corporation, Japan). The temperature of the column was programmed from 120 to 260 °C at 7 °C/min and 260 to 370 °C at 15 °C/min. The temperature of the vaporizing chamber, interface, and ion source was 200 °C. Helium was used as carrier gas (1·21 ml/min). FAMEs were identified by the comparison between the measurement data (fragment ion) and the database (NIST05, Shimadzu Corporation, Japan).

Histopathology of host skin infected with N. girellae

Twenty uninfected *S. dumerili* specimens, weighing ~70 g each, were divided into 2 groups and each group was transferred to a 200 l tank. The fish were acclimatized for a week. The tank was aerated and supplied with seawater. The fish were fed a 2·5 mm pellet diet twice a day and the daily feeding rate was 3% of body weight. The temperature was maintained at 25·5 ± 0·5 °C using a heating device throughout the trial. After acclimatization, ~3200 *N. girellae* oncomiracidia were placed into the tank (infected group) while the seawater supply was discontinued for 1 h to assist in establishment of the infection. No oncomiracidia were put into the other tank (non-infected group). At 12 days after exposure to the parasites, the fish were sampled. Skins from the belly including the attached *N. girellae* were dissected from the sampled fish. The fish in the non-infected group were also sampled and skins from the belly were dissected. The dissected skin measured 5 mm^3. The skin samples were fixed in Bouin's solution. All of the samples were processed using a standard histological technique, embedded in paraffin wax, sectioned at 5 μm thickness and stained with haematoxylin and eosin (H & E). Histological sections were observed under a light microscope.

RESULTS

Analysis of lipid content and fatty acid composition of adult N. girellae, S. dumerili *skin and cutaneous mucus* (*Experiment 1*)

Lipid content and fatty acid composition of adult *N. girellae*, *S. dumerili* skin and cutaneous mucus in the group not fed the diet supplemented with [^{13}C] fatty acids were analysed to compare the values from the parasites and their host. The lipid content was 0·8 mg/g for adult *N. girellae* (body length: 4·12 ± 0·24 mm, number of parasites per fish: 63·1 ± 15·2; mean ± S.D.), 3·4 mg/g for *S. dumerili* skin and 5·4 mg/g for *S. dumerili* cutaneous mucus. *N. girellae* had a qualitative fatty acid profile similar to its host (Fig. 1). The parasites and *S. dumerili* skin and mucus had a relatively high weight-percentage of C16 : 0, C18 : 1(n-9), C18 : 0, C20 : 5(n-3) and C22 : 6(n-3) fatty acids. The percentages of most fatty acids analysed from *N. girellae* tended to be lower than those from *S. dumerili* skin and mucus but *N. girellae* contained a significantly higher percentage of C22 : 6(n-3) and C22 : 5(n-6) fatty acids compared with *S. dumerili* skin and mucus. The percentages of most fatty acids from *S. dumerili* cutaneous mucus also tended to be lower than the skin but the mucus contained a significantly higher percentage of C22 : 1(n-9) fatty acid.

In addition, the origin of the parasite fatty acids was investigated by analysing the parasites grown on *S. dumerili* fed a diet supplemented with ^{13}C-labelled fatty acids. [^{13}C] fatty acids [C16 : 0, C18 : 2(n-6) and C18 : 1(n-9)] were detected in *S. dumerili* skin when *S. dumerili* were fed the diet supplemented with [^{13}C]

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Table 2. Percentage of total free [^{12}C] fatty acids and [^{13}C] fatty acids from adult *Neobenedenia girellae*, *Seriola sumerili* skin and mucus

	Skin		Mucus		*N. girellae*	
Fatty acids	[^{12}C] Fatty acids	[^{13}C] Fatty acids	[^{12}C] Fatty acids	[^{13}C] Fatty acids	[^{12}C] Fatty acids	[^{13}C] Fatty acids
C14 : 0	1·84	0·00	2·50	0·00	1·45	0·00
C15 : 0	0·54	0·00	1·27	0·00	0·62	0·00
C16 : 1(n-9)	2·57	0·00	1·99	0·00	1·68	0·00
C16 : 1(n-7)	0·12	0·00	0·00	0·00	0·64	0·00
C16 : 0	24·44	0·75	18·97	0·00	21·66	0·67
C17 : 1(n-9)	0·79	0·00	0·84	0·00	0·48	0·00
C17 : 0	3·90	0·00	2·32	0·00	2·51	0·00
C18 : 3(n-3)	0·20	0·00	0·00	0·00	0·00	0·00
C18 : 2(n-6)	4·15	0·37	3·16	0·00	2·37	0·00
C18 : 1(n-9)	12·04	0·96	11·67	0·00	7·93	0·00
C18 : 1(n-7)	3·04	0·00	3·42	0·00	2·28	0·00
C18 : 0	10·59	0·00	8·77	0·00	7·15	0·00
C20 : 5(n-3)	8·97	0·00	4·38	0·00	7·44	0·00
C20 : 4(n-6)	2·97	0·00	1·91	0·00	2·81	0·00
C20 : 2(n-6)	0·27	0·00	0·97	0·00	0·54	0·00
C20 : 1(n-9)	1·82	0·00	2·43	0·00	1·09	0·00
C20 : 0	0·32	0·00	1·44	0·00	1·35	0·00
C22 : 6(n-3)	16·96	0·00	10·33	0·00	23·53	0·00
C22 : 5(n-6)	2·62	0·00	2·63	0·00	5·95	0·00
C22 : 1(n-9)	0·78	0·00	14·84	0·00	2·60	0·00
C22 : 0	0·06	0·00	2·05	0·00	2·01	0·00
C24 : 1(n-9)	0·15	0·00	0·00	0·00	1·77	0·00
C24 : 0	0·14	0·00	4·10	0·00	1·47	0·00

fatty acids (Table 2). However, no [^{13}C] fatty acids were detected in the *S. dumerili* mucus. On the other hand, ^{13}C-labelled C16 : 0 was detected from adult *N. girellae* (body length: 4·10 ± 0·26 mm, number of parasites per fish: 62·6 ± 18·0) collected from *S. dumerili* that were fed the diet supplemented with [^{13}C] fatty acids. The ratio of [^{13}C]16 : 0 to [^{12}C]16 : 0 for *N. girellae* was 0·03 : 1; this ratio was the same as that for *S. dumerili* skin. The fatty acid profile was similar to previous analyses of each sample collected from fish that were fed the basal diet without [^{13}C] fatty acids.

Analysis of lipid content and fatty acid composition of adult N. girellae*, skin and cutaneous mucus collected from two host fish species* (*Experiment 2*)

The number and size of the parasites grown on 2 host fish species, *S. dumerili* and *V. variegates* were compared. *S. dumerili* was more susceptible to *N. girellae* than was *V. variegatus*. The number of *N. girellae* per cm^2 on *S. dumerili* was significantly higher than on *V. variegatus* (Table 3). Also, the length of the parasites from *S. dumerili* was significantly greater than that of parasites on *V. variegatus*.

The lipid content was analysed for adult *N. girellae* on *S. dumerili*, adult *N. girellae* on *V. variegatus*, *S. dumerili* skin, *V. variegatus* skin, *S. dumerili* cutaneous mucus and *V. variegatus* cutaneous mucus. The lipid content of each sample was 0·9 mg/g, 0·6 mg/g, 3·9 mg/g, 5·1 mg/g, 6·2 mg/g and 6·6 mg/g, respectively. The fatty acid profiles of skin, cutaneous mucus and *N. girellae* collected from *V. variegatus* were similar, qualitatively and quantitatively, to those of *S. dumerili* (Fig. 2). *N. girellae* collected from both host fish species had a relatively high weight-percentage of C16 : 0, C18 : 1(n-9), C18 : 0, C20 : 4(n-6) and C22 : 6(n-3) fatty acids. However, the percentage of C16 : 0 fatty acid of *N. girellae* collected from *S. dumerili* tended to be higher than that collected from *V. variegatus*. C16 : 0 fatty acid of both *S. dumerili* skin and cutaneous mucus also tended to be higher than that of *V. variegatus*. The percentage of C18 : 0 and C20 : 4(n-6) fatty acids of *N. girellae* collected from *S. dumerili* tended to be lower than that collected from *V. variegatus*.

Histopathology of host skin infected with N. girellae

The thickness of *S. dumerili* epidermis with attached *N. girellae* in the infected group was markedly thin compared with that of non-infected fish (Fig. 3A and B). Mucus cells were observed in the epidermis of non-infected fish (Fig. 3C). On the other hand, mucus cells in the epidermis with attached *N. girellae* were rarely observed (Fig. 3D).

DISCUSSION

Lipids of endoparasitic parasites play a vital role in the long-term survival and completion of their

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Table 3. Susceptibility of amberjack *Seriola dumerili* and spotted halibut *Verasper variegatus* to infection by *Neobenedenia girellae*

Tested fishes	No. of parasites per fish	No. of parasites per cm^2 per fish surface area	Parasite body length (mm)
S. dumerili	241·2 ± 25·4	0·75 ± 0·08*	4·01 ± 0·38**
V. variegatus	235·8 ± 52·9	0·45 ± 0·22	3·33 ± 0·62

Values are mean and standard deviations.
Asterisk indicates a significant difference between values for *S. dumerili* and *V. variegatus*. (* $P<0·05$, ** $P<0·01$).

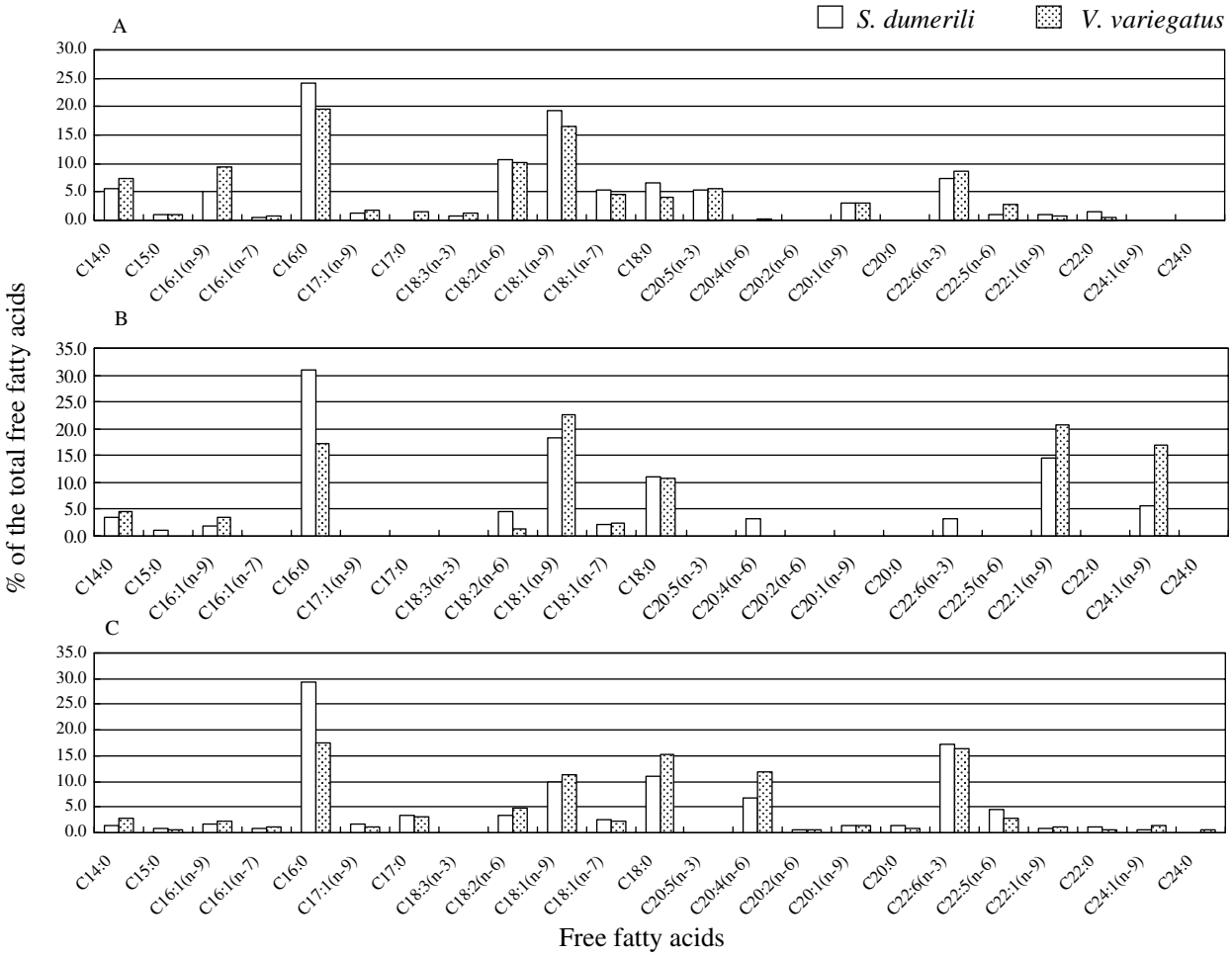

Fig. 2. Fatty acid composition of adult *Neobenedenia girellae* (C) and of (A) the skin and (B) the cutaneous mucus of the two host species *S. dumerili* and *V. variegatus*.

life cycles (Furlong, 1991; Sherman, 1998). Some protozoans have a fatty acid composition reflecting that of their hosts or the cultivation medium (Dixon and Williamson, 1970). The findings regarding *Plasmodium falciparum* fatty acid metabolism have been useful for developing a cultivation medium (Mi-ichi *et al*. 2006). In addition, characterization of parasite fatty acid metabolism was important for developing a new strategy for controlling malaria (Mi-ichi *et al*. 2006). However, the lipid content and fatty acid composition of monogenean parasites have not been investigated.

The lipid content of adult *N. girellae* was ~0·8 mg/g and was less than one-fourth that of *S. dumerili* skin and mucus. *N. girellae* had a relatively high weight-percentage of C16 : 0 palmitic, C18 : 1(n-9) oleic, C18 : 0 stearic and C22 : 6(n-3) docosahexaenoic fatty acids among analyses in triplicate. In particular, the percentage of both C16 : 0 and C22 : 6(n-3) fatty acids accounted for more than 20% of the total fatty acids. In *P. falciparum*, C16 : 0, C18 : 1(n-9) fatty acids are necessary for complete cell-cycle progression and intraerythrocytic development (Mitamura *et al*. 2000). The fatty acid profile

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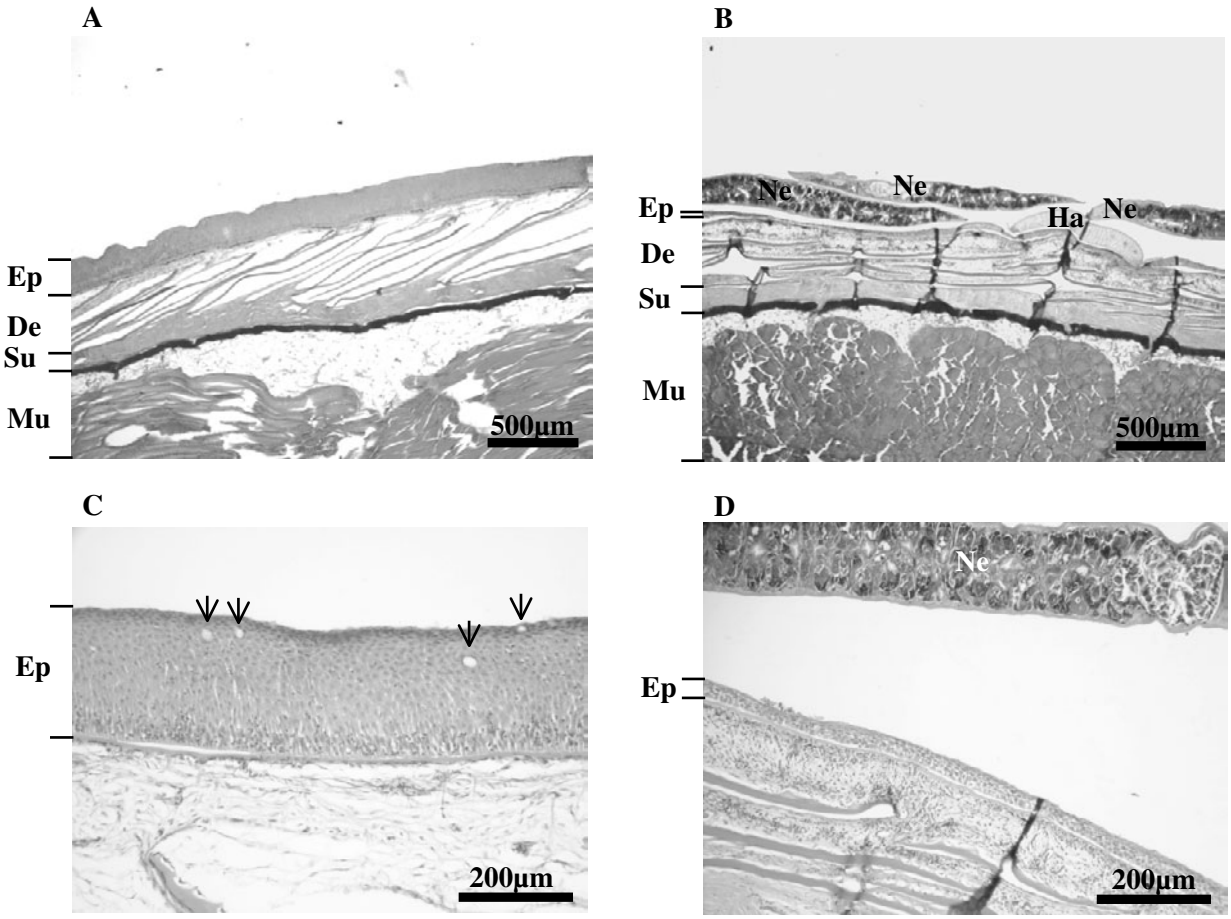

Fig. 3. Histological sections of the skin of the amberjack *Seriola dumerili* non-infected (A and C) and infected with *Neobenedenia girellae* (B and D). The arrow in (C) indicates mucus cell. Ep, epidermis; De, dermis; Su, subcutaneous tissue; Mu, muscle; Ne, adult *N. girellae*; Ha, haptor.

of *Perkinsus marinus*, which is an oyster protozoan parasite, consisted primarily of C16 : 0, C20 : 4(n-6), C20 : 5(n-3) and C22 : 6(n-3) fatty acids, and the percentage of both C16 : 0 and C22 : 6(n-3) fatty acids accounted for more than 15% of the total fatty acids (Soudant and Chu, 2001). Thus the fatty acids that are relatively abundant in *N. girellae* also occur in parasitic protozoans, where they are important for the completion of their life cycles. More studies are needed to determine whether these fatty acids are important for *N. girellae* growth, survival and completion of their life cycle.

C16 : 0, C18 : 2(n-6) and C18 : 1(n-9) containing ^{13}C were detected from *S. dumerili* skin when *S. dumerili* were fed a diet supplemented with [^{13}C] fatty acids, but no [^{13}C] fatty acids were detected from the *S. dumerili* cutaneous mucus. On the other hand, ^{13}C-labelled C16 : 0 was detected from adult *N. girellae* collected from *S. dumerili* which were fed the diet supplemented with [^{13}C] fatty acids. The ratio of [^{13}C] 16 : 0 to [^{12}C] 16 : 0 in *N. girellae* was the same as that in *S. dumerili* skin and the percentage of C16 : 0 acid in *N. girellae* was similar to that in *S. dumerili* skin. Cutaneous mucus from both host fish species *S. dumerili* and *V. variegatus* contained a significantly higher percentage of C22 : 1(n-9) fatty acid, but adult *N. girellae* did not contain a high level of this fatty acid. In addition, the thickness of *S. dumerili* epidermis attached with *N. girellae* was markedly thin. Furthermore, mucus cells in epidermis attached with *N. girellae* were rarely observed, suggesting that cutaneous mucus production in the skin surface site of *N. girellae* infection could be low. These results suggest that *N. girellae* feeds primarily on host epithelial cells, and that it may be possible to use established fish cell lines (Uenokawa and Shirahata, 2000) for cultivation of *N. girellae*. *In vitro* techniques reduce the laboratory space required and eliminate the need to use and maintain living host fish. Continuous propagation of the parasite *in vivo* requires considerable space and supply of healthy host fish. This study shows that *S. dumerili* epidermis became thin due to *N. girellae* infections. Thus, the host skin would tend to be easily bruised when the fish rub against the net. Fish skin mucus is an important defence barrier against fish pathogens and contains enzymes and antibodies that can kill invading organisms (Shephard, 1994). Epidermal mucus cells produce skin cutaneous mucus constantly and the production of a large amount of

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cutaneous mucus enhances pathogen removal (Willoughby, 1971, 1972). This suggests that metabolism including fatty acid metabolism of the epidermal mucus cells may be fast, and thus [^{13}C] fatty acids were not detected from the *S. dumerili* cutaneous mucus produced by epidermal cells. In addition, infections with large populations of capsalid monogeneans may cause mucus hyperproduction of host fish (Paperna, 1991) and the metabolism of the epidermal mucous cells of fish infected with the parasites may be faster than that of fish without parasitic infection. The other possibility is that mucus may be pre-formed in mucus cells of which the granules are not labelled.

Seriola dumerili was more susceptible to *N. girellae* than *V. variegatus* under our conditions, although these fishes were exposed to oncomiracidia in separate aquaria. The parasites were larger and more abundant on *S. dumerili* than on *V. variegatus*. The fatty acid profiles of skin, cutaneous mucus and *N. girellae* collected from *V. variegatus* were similar, qualitatively and quantitatively, to those of *S. dumerili*, suggesting that the fatty acid composition of both host species may not be responsible for the difference in susceptibility between *S. dumerili* and *V. variegatus* or promote *N. girellae* growth. Host skin lipids act as host-finding signals for trematode *Fasciola hepatica* miracidia (Wilson and Denison, 1970) and stimulate the attachment and penetration of trematode *Trichobilharzia ocellata* cercariae (Haas and Van de Roemer, 1998). Skin-surface lipids of the host with their higher fatty acid contents stimulate higher cercarial penetration rates, and fatty acids as penetration stimuli may offer advantages for *T. ocellata* cercariae by increasing the specificity for an invasion of terrestrial vertebrates (Haas and Van de Roemer, 1998). The percentage of C16 : 0 fatty acid of *N. girellae*, skin and cutaneous mucus collected from *S. dumerili* tended to be higher than that collected from *V. variegatus* and, thus, more studies are needed to examine the role of C16 : 0 in host susceptibility to *N. girellae*.

In this study, adult *N. girellae* and *S. dumerili* skin and mucus had a high weight-percentage of C16 : 0, C18 : 1(n-9), C18 : 0 and C22 : 6(n-3) fatty acids. [^{13}C] fatty acids were detected in *S. dumerili* skin and adult *N. girellae* on *S. dumerili* when *S. dumerili* were fed the diet supplemented with [^{13}C] fatty acids, but no [^{13}C] fatty acids were detected from the *S. dumerili* cutaneous mucus. In addition, thickness of *S. dumerili* epidermis attached with *N. girellae* was markedly thin. These results suggest that *N. girellae* feeds primarily on host epithelial cells. Furthermore, the fatty acid profile of skin, cutaneous mucus and *N. girellae* collected from *V. variegatus* was similar, qualitatively and quantitatively, to that of *S. dumerili* which was more susceptible to the parasite. This suggests that fatty acid composition of both host species may not be involved in susceptibility and *N. girellae* growth between *S. dumerili* and *V. variegatus*. Together, the results in this study may be useful to develop methods for *N. girellae* cultivation *in vitro*, of which culture methods are needed to further investigate fatty acid metabolism so that new strategies for controlling *N. girellae* can be developed, and may ultimately serve to develop new strategies to control *N. girellae* infections.

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