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Sea lice infections of wild fishes near ranched southern bluefin tuna (*Thunnus maccoyii*) in South Australia

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ABSTRACT

In contrast with sea lice infestations of other farmed fishes, attached larval stages of sea lice on ranched southern bluefin tuna (*Thunnus maccoyii*) are rarely detected. In this study, we monitored sea lice on ranched *T. maccoyii* and surveyed wild fishes adjacent to ranching sea cages over a 3-month period in early 2009. Prevalence of the adult *Caligus chiastos* on tuna within a day of arrival at the ranching site was 10%; prevalence then increased significantly and peaked almost 25 days later to 75%; by harvest (after a further 18–28 days), prevalence decreased significantly to 0%. We collected and examined a total of 502 wild fishes outside *T. maccoyii* sea cages, comprising 307 Degen's leatherjackets (*Thamnaconus degeni*), 136 yellowtail horse mackerel (*Trachurus novaezelandiae*), 31 sand trevally (*Pseudocaranx wrighti*), 10 West Australian salmon (*Arripis truttacea*), 6 Port Jackson sharks (*Heterodontus portusjacksoni*), and a single blue mackerel (*Scomber australasicus*) and pilchard (*Sardinops sagax*); we also examined an additional 10 pilchards that were collected from the centre of Spencer Gulf and stored fresh in a *T. maccoyii* feed bin. Of these potential hosts, we identified adult *C. chiastos* only from Degen's leatherjackets; of the many larvae also occurring on this host, molecular comparison of five specimens analysing cytochrome *C* oxidase I region of mitochondrial DNA and five specimens analysing partial D1–D2 domains of 28S rDNA confirmed that these were *C. chiastos*. In contrast with the decline in infections of *C. chiastos* on ranched *T. maccoyii* near the end of March, on Degen's leatherjackets there was a significant increase in prevalence and abundance over the study period, with a peak prevalence of 97.14% and a mean abundance reaching 11.17 lice per fish near the end of April. The percentage of chalimus larvae on Degen's leatherjackets increased over the study period, ranging from 0% near the start of sampling to over 93% on the final sample date. We also recorded additional copepod infestations, including *Orbitacolax williamsi* on Degen's leatherjackets, *Caligus* sp. on sand trevally, and *Dissonus nudiventris* on Port Jackson sharks. We conclude that Degen's leatherjacket, which is a major scavenger of excess tuna feed, is likely to contribute to sea lice infestations of *T. maccoyii*.

1. Introduction

Sea lice are a significant pathogen in marine finfish farming (Rosenberg, 2008). Fish farmed in sea cages are likely to be infested by parasites from wild fishes and because of their high concentration can in turn become a source of parasites (Costello, 2009). Epizootics of sea lice (predominantly *Caligus chiastos*) have recently been observed to occur on southern bluefin tuna (*Thunnus maccoyii*) ranched off Port Lincoln, South Australia (Hayward et al., 2008, 2009). This species has also been recorded on a number of other aquacultured species of fishes, including mulloway (*Argyrosomus japonicus*) and yellowtail kingfish (*Seriola lalandi*) in Australia (Hayward et al., 2007), and John's snapper (*Lutjanus johni*) in Malaysia (Venmathi Maran et al., 2009). On *T. maccoyii*, the numbers of these lice have been demonstrated to be correlated with two indicators of stress — plasma cortisol and glucose — as well as with low condition index and gross eye damage (Hayward et al., 2008, 2009, 2010). In contrast with sea lice infestations of other farmed fishes, attached larval stages of *C. chiastos* on ranched *T. maccoyii* are rarely detected. For example, of over 5400 individual lice collected from ranched tuna in 2008, only three (0.06%) were larval stages; the remainder were adult females and males of predominantly *C. chiastos* (Hayward et al., submitted for publication). This indicates that infected wild fishes attracted to tuna sea cages must be the source of infections of mobile, adult *C. chiastos*. In this study, we therefore aimed to monitor numbers of *C. chiastos* on

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Table 1
Dates of monitoring sea lice burdens on ranched southern bluefin tuna (*Thunnus maccoyii*) off Port Lincoln in early 2009 (LCF, length to caudal fork).

Dates of sampling	Sea cage no.	No. of tuna	Tuna mean LCF (cm)
01 Feb	'tow'	40	93.1 (78–105)
17 Feb	1–4	8	97.6 (92–107)
26 Feb	3, 4	4	107.8 (100–115)
12 Mar	3	3	115.3 (115–116)
16 Mar	3	30	107.7 (89–140)
23 Mar	3	30	111.3 (103–126)

ranched tuna from transfer to the pens at the beginning of the season through to harvest, and to sample wild fishes around ranching sea cages over the same period, to determine which species are likely to be the main host(s) of chalimus stages.

2. Materials and methods

2.1. Sampling of T. maccoyii

Two samples each of 10 *T. maccoyii* from different schools caught in the Great Australian Bight by a tuna ranching company were examined for sea lice in late January 2009.

A tow cage containing *T. maccoyii* caught in the Great Australian Bight (33°47.81S 132°145.617E) on 19 January 2009 by a second tuna ranching company arrived at the ranching zone on the evening of 31 January 2009. On the morning of 1 February 2009, a sample of 40 of these tuna were weighed and measured; these tuna were also examined for sea lice and returned to the tow cage. These tuna were then transferred into four ranching sea cages (each of 40 m diameter), two on the same day and the other two the following day. Feeding of the tuna commenced on 2 February 2009 (three sea cages) and 3 February (one sea cage). Samples of tuna from these four sea cages were examined on several dates, up until the time of harvest; Table 1 lists the dates and sample sizes in this study.

All lice visible to the naked eye were collected as soon as possible at the time of capture of tuna; any additional lice remaining on tuna surfaces were then detected using a technique described in Hayward et al. (2010), in which wetted fingers were gently moved over all the external skin surfaces of each tuna, to feel for characteristic hard 'bumps' (indicating the presence of sea lice obscured by tuna mucus and, in rare cases, attached chalimi). All lice were collected and preserved in ethanol and identified later in the laboratory using a dissecting microscope.

2.2. Sampling of wild fishes beside T. maccoyii ranching sea cages

Wild fishes were sampled from outside these four tuna ranching sea cages using a gill net and two fish traps. Table 2 lists the dates of collection, the species, and their numbers. With the exception of Port Jackson sharks (which were examined freshly without placement in plastic bags), all fish were placed individually as quickly as possible after catching into ziplock plastic bags; 100% ethanol was added, and the bags were sealed. The bagged fishes were stored on ice until they were returned to the laboratory, where they were transferred to a −20°C freezer until examination. The external surfaces of these fishes and the contents of the bags were examined under a dissection microscope; all lice detected were collected and stored in 100% ethanol. Adult lice were identified to species under low- and high-power microscopes; samples of larval lice were identified using molecular methods (see below).

2.3. Data analysis

Parasite infections were characterised, for each species of host, by prevalence (the number of host infections as a proportion of the population at risk) and mean abundance (the average number of parasites in all hosts; Bush et al., 1997). Sterne's exact 95% confidence intervals were calculated for prevalence, and 95% bootstrap confidence intervals (with 2000 replications) were calculated for mean abundances, using the statistical package 'Quantitative Parasitology 3.0' (Reiczigel and Rózsa, 2005). Prevalences and mean abundance for each species for each sample date were compared pairwised with other sample dates. Given the high total number of pairwise comparisons, an alpha level of 0.01 was regarded as significant for these statistics. Spearman's rank correlation coefficient was calculated for the relationship between *Caligus* counts and host length using the VassarStats online statistical calculator (http://www.faculty.vassar.edu/lowry/VassarStats.html); a Mann–Whitney *U* test was used to compare the total numbers of sea lice on male and female hosts.

2.4. Molecular comparisons of parasites

Genomic DNA extraction from individual parasites (an adult male, an adult female and five chalimus larvae) was performed with QIAamp DNA Mini Kit (Qiagen Inc., Valencia, CA, USA) following the manufacturer's protocol. Polymerase chain reaction to amplify the mitochondrial COI region was conducted mainly according to Øines and Heuch (2005). Briefly, 5 μl of the extracted DNA and 75 μl of water

Table 2
Numbers of wild species collected from beside tuna ranching sea cages in early 2009 and their external (skin and fin) parasites (excluding *Caligus chiastos* from *Thamnaconus degeni*).

Species abundance	*n*	Date	LCF (cm): mean (range)	Parasites	Prevalence (%)	Mean
Thamnaconus degeni	28	14 Feb	11.55 (8.8–13.7)	*Orbitacolax williamsi*	17.9	0.21
	19	20 Feb	10.99 (8.5–13.5)	*O. williamsi*	31.6	0.32
	55	12 Mar	10.22 (7.4–13.5)	*O. williamsi*	52.7	0.84
	23	24 Mar	11.46 (8.5–13.7)	*O. williamsi*	73.9	1.30
	101	01 Apr	10.15 (7.0–14.2)	*O. williamsi*	70.3	1.41
	44	08 Apr	10.59 (8.1–13.2)	*O. williamsi*	86.4	1.73
	35	21 Apr	10.15 (7.4–12.8)	*O. williamsi*	60.0	1.31
Pseudocaranx wrighti	30	04 Feb	16.9 (16.0–18.5)	*Caligus* sp.	50.0	0.83
	1	14 Feb	8.5	*Caligus* sp.	100	1.00
Heterodontus portusjacksoni	6	05 Feb	57.6 (48.0–61.5)	*Dissonus nudiventris*	100	3.67
Sardinops sagax	10	11 Feb	14.5 (13.8–15.0)	–	–	–
	1	14 Feb	23.4	–	–	–
Scomber australasicus	1	14 Feb	23.5	–	–	–
Trachurus novaezelandiae	22	14 Feb	21.6 (17.5–23.9)	–	–	–
	71	20 Feb	21.4(19.5–25.0)	–	–	–
	26	24 Mar	21.0 (18.5–24.5)	–	–	–
	15	08 Apr	21.1(19.6–23.4)	–	–	–
	3	21 Apr	20.7(20.5–20.9)	–	–	–
Arripis truttacea	8	20 Feb	16.8 (16.0–18.0)	–	–	–
	2	21 Apr	23.0 (22.4–23.6)	–	–	–