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SYMBIONTS OF BIGEYE AND YELLOWFIN TUNA AS POTENTIAL STOCK MARKERS FOR TUNA IN INDONESIA ARCHIPELAGIC WATERS

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ABSTRACT

The purpose of this research was to obtain data on the symbionts of bigeye (*Thunnus obesus*) and yellowfin tuna (*Thunnus albacares*) to assess them as potential stock markers to reveal migration patterns important in fisheries management. We collected bigeye and yellowfin 28-48 cm fork length from nine locations in several provinces in Indonesia. Gills (filaments and branchial arches), stomach wall, liver, pyloric caeca and intestine were examined by separating the tissue under a dissecting microscope and symbionts found were preserved in 70% alcohol. The result showed that 7 types of didymozoids (3 *Didymosulcus* spp. and 4 *Kollikeria* spp.) and an acanthocephalan (*Bolbosoma* sp.) could be suitable biological tags. This result will help to develop stock assessment in fisheries management to maintain the population of tuna in Indonesia.

KEYWORDS : Indonesia, Symbionts, Stock marker, Tuna

INTRODUCTION

Indonesia's pelagic fisheries resources are of high importance to the nation's economy and as a domestic food resource. Two species of critical importance to Indonesia and to country neighbours in the Indian Ocean (IO) and Western and Central Pacific Ocean (WCPO) regions are yellowfin tuna (YFT), *Thunnus maccoyii,* and bigeye tuna (BET), *T.obesus.* Most recent stock assessments for these species suggest YFT in both region and BET in the IO are currently not overfished, but that BET in WCPO are in an overfished state and current catches are unsustainable (Davies et al 2014, Harley et al 2014, IOTC-SC 16 2013). However, the assessment are weakened by insufficient clarity of stock structure. Assessments to date are based on assumptions of single panmictic spawning populations of YFT and BET within the IO and WCPO, but some recent studies suggest there may be distinct metapopulations across the range (Dammannagoda et al 2008, Nugraha et al 2011, Wells et al 2011, Fraile et al 2013, Swaraj et al 2013). Analyses of data from tuna tagging programs in the IO and WCPO also suggest that movement/mixing rates of YFT and BET may not be as high as previously thought (Hoyle et al 2013). If populations are distinct, or mixing rates are very low within a panmictic population, some population (or sub-regions) could be susceptible to local over-exploitation and ill-informed management decisions.

The use of parasites as biological tags in population studies of marine fish is becoming popular (MacKenzie et al., 2008) because parasite data are relatively inexpensive to collect and the method forms a useful component in a multidisciplinary approach to stock identification (Lester, 1990; Begg and Waldman, 1999). The principle is that where the parasite fauna of fish from two areas is the same, the fish either have grown in a similar environment or have a common history. Where the fauna is different, the history of the fish is different according to the time scale of the parasite counted: recent history for temporary parasites, long-term history for

permanent parasites (Lester, 1990). Methods used to analyse parasite data are becoming increasingly sophisticated (MacKenzie and Abaunza, 1998; Perdiguero-Alonso et al., 2008).

The Didymozoidae is a major family of trematodes which have radiated in pelagic fishes, especially tunas (Scombridae) (Pozdnyakov &Gibson, 2008). Adult didymozoids occur principally in the tissues of their hosts rather than in the gut lumen. They must therefore be sought specifically throughthe examination of the tissues where they reside. Another key feature of the family is that the adults retain eggs in the uterus (typically, it is thought, to be dispersed only when the parasite dies), which has the effect of making most didymozoids bright yellow. Many didymozoids form pairs and in some cases there is sexual differentiation into males and females; it is best to keep such pairs together and separate from other individuals so that they can be described as a pair. Many didymozoids are thread-like and may reach lengths that exceed a metre (e.g. Noble, 1975). Such species are challenging to work with. They can only be collected by slow and painstaking dissection of the surrounding tissues. A special effort has to be made to find the anterior end of the parasite, because identification is generally impossible without it.

This research is part of ACIAR Project FIS/2009/059: *Developing research capacity for management of Indonesia's pelagic fisheries resources* in which characterizations of parasites, genetics and otolith chemistry are being used in a holistic approach to examine the population structure and rates of mixing of YFT and BET across the Indonesian archipelago and connectivity to populations in adjacent ocean regions. Here we describe progress with identifying potentially useful parasite markers.

MATERIALS AND METHODS

Bigeye and Yellowfin Tuna were collected from 9 sites ; Padang, Prigi, Palabuhanratu, Bitung, Gorontalo, Kendari, Ambon, Sorong, and Jayapura in Indonesia (Fig 1.). They were obtained directly from fishermen or from local fish markets when the origin of the fish was known. Immediately after capture, the gills and viscera were removed, placed into individual

plastic bags with a label giving site location, date and time of capture and caudal fork length (LF), and frozen for later laboratory examination. Additional samples were collected from the Maldives and from the Solomon Islands.

Dissections of gills and stomachs were carried out according to the methods of Lester *et al.* (2001).After scanning the external surface, the gill cover was removed and the gills taken out by dorsal and ventral cuts. The gill arches were opened and the external and internal gill surfaces examined under a dissecting microscope to find didymozoids. The viscera were separated into stomach, pyloric caeca, intestine and liver. It was best to open each of these organs separately in an appropriately-sized petri dish and to examine it progressively under a dissecting microscope electron. Separation of organs allowed for a cleaner examination and also for the accurate recording of the site of infection. Any parasites found were removed and preserved in 70% alcohol.

Figure 1. A map showing the Indonesian sampling locations: (1) Padang, (2) Prigi, (3) Palabuhanratu, (4) Gorontalo, (5) Kendari, (6) Bitung, (7) Ambon, (8) Sorong, and (9) Jayapura. (Modified from Google Maps).

RESULTS

Ten types of parasites were found: 3 types of *Didymosulcus*, 4 types of *Kollikeria*, *Hirudinella ventricosa*, *Bolbosoma sp.* and *Rhadinorhynchus sp*. (Tables 1 and 2)

Of the 10 types eight appear to be suitable as biological tags. These include the 7 didymozoids (*Didymosulcus* spp. and *Kollikeria* spp.), and an acanthocephalan (*Bolbosoma* sp.).

Table 1. Parasites found in big-eye tuna

BIGEYE TUNA (*Thunnus obesus*)		
Parasites	**Type**	**Organ**
Didymosulcus sp.	type 1	Gill
Didymosulcus sp.	type 2	Gill
Didymosulcus sp.	type 3	Gill
Hirudinella ventricosa		Stomach
Kollikeria sp.	type 1	Stomach wall
Bolbosoma sp.		Stomach wall
Rhadinorhynchus sp.		Stomach wall
Kollikeria sp.	type 2	Liver
Kollikeria sp.	type 3	Pyloric caeca
Kollikeria sp.	type 4	Intestine wall
Rhadinorhynchus sp.		Intestine wall
Bolbosoma sp.		Intestine wall

Table 2. Parasites found in yellowfin tuna

YELLOWFIN TUNA (*Thunnus albacares*)		
Parasites	**Type**	**Organ**
Didymosulcus sp.	type 1	Gill
Didymosulcus sp.	type 2	Gill
Didymosulcus sp.	type 3	Gill
Hirudinella ventricosa		Stomach
Kollikeria sp.	type 1	Stomach wall
Bolbosoma sp.		Stomach wall
Rhadinorhynchus sp.		Stomach wall
Kollikeria sp.	type 2	Liver
Kollikeria sp.	type 3	Pyloric caeca
Kollikeria sp.	type 4	Intestine wall
Rhadinorhynchus sp.		Intestine wall
Bolbosoma sp.		Intestine wall

The 3 types of *Didymosulcus* were from gill arch and filament. *Didymosulcus* type 1 was comma shaped and occurred in pairs embedded in the filaments of a gill arch (Fig. 2). Type 2 were elongated (Fig. 3) and Type 3 was more rounded (Fig 4).

	
Fig.2 *Didymosulcus* type 1, a didymozoid in the gills	Fig.3 *Didymosulcus* type 2, a didymozoid in the gills
	
Fig.4 *Didymosulcus* type 3, a didymozoid from the gills	Fig.5 *Kollikeria* type 1, a didymozoid from the stomach wall
	
Fig.6 *Kollikeria* type 2, a didymozoid in the liver	Fig.7 *Kollikeria* type 3, a didymozoid from the pyloric caeca

	
Fig.8 *Kollikeria* type 4, a didymozoid from the intestine wall	Fig.9 Giant worm, *Hirudinella ventricosa* from the stomach
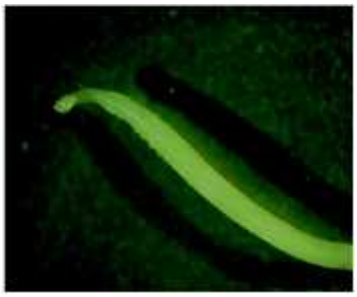	
fig.10 Adult *Rhadinorhynchus* sp. from the intestinal lumen	fig.10 Juvenile *Bolbosoma*sp. from wall of intestine.

Four types of *Kollikeria* were found in the viscera. Type 1 found in the stomach wall was a globular female within which was a small male (figure 5). Type 2 was generally found in groups in the liver (Fig6). Type 3 was a large species with an elongate shape found in the pyloric caeca (Fig7). Type 4 was a small species found in the intestinal wall (Fig.8).

Besides the didymozoids we also found the giant worm *Hirudinella ventricosa* (Fig. 9) in stomach and 2 genera of Acanthocephalan. Adult *Rhadinorhynchus sp.* were found in the intestinal lumen (Fig.10). Immature *Bolbosoma* sp. were chiefly embedded in the wall of the intestine (Fig. 11). They had a distinct bulge near the proboscis covered with abroad band of small hooks. A second band occurred on the body between the bulge and the proboscis. The

species may be *Bolbosoma vasculosum* which are commonly found in the northern Atlantic, North Pacific and Mediterranean (Williams and Williams, 1996).

DISCUSSION

The correct identities of the parasites are being investigated. It is not yet known whether the same species of parasites occur in both species of tuna, or indeed whether the same species of didymozoid occurs in different places in the same fish. Further morphological examination is underway to be followed by DNA sequencing

The genus *Didymosulcus*is specific to the family Scombridae and almost specific to tunas. They are considered a potentially dangerous parasite of tunas, little tunas and mackerels around the world (Williams and Williams, 1996).

Didymozoids such as is *Didymosulcus* and *Kollikeria* spp. are thought to release eggs and degenerate when the host fish matures. The probable life cycle involves eggs released from fish being eaten by a pelagic snail. Tiny cercaria develop in the snail and are eventually released into sea water. A cercaria is thought to be eaten by a planktonic copepod, the copepod eaten by small fish and then the small fish is eaten by a tuna.

Seven types of didymozoids and the acanthocephalan *Bolbosoma* sp. were embedded in host tissue and were considered suitable as biological tags. The use of parasites as biological tags for identification and definition of fish stocks have often been used because during such migrations fishes are accompanied by their parasites (Sinderman, 1961; Gibson, 1972; Wickings and MacFarlane, 1973; Mackenzie, 1983, 1986; McGladdery and Burt, 1985; Butorina and Shedko, 1989; Mackenzie, 1990; Moser and Hsieh, 1992). As noted by Bailey et al. (1988) and Lester (1990), the geographical variation of the distribution and abundance of parasites is an excellent source of information of movements and migration of marine fishes to know the population of fish.

The parasites chosen for future analysis were embedded in the tissues rather than free in the lumen of the gut. Those in the lumen were thought to be temporary compared with parasites in tissues. Other stock discrimination studies have focused on juvenile anisakid nematodes or juvenile trypanorhynch cestodes. These were rare or absent from the fish we dissected. Didymozoids are thought to release eggs and degenerate when the host fish matures. As all the tuna we examined were juveniles it is likely we can assume that once infected the fish stayed infected until they were caught. Thus they can be considered long-term markers for our statistical analyses.

CONCLUSSION

Seven types of didymozoids (3 *Didymosulcus* spp. and 4 *Kollikeria* spp.) and an acanthocephalan (*Bolbosoma* sp.) could be suitable biological tags. Need more research about symbionts as biological tags to develop stock assessment in fisheries management to maintain the population of tuna in Indonesia. This research will be continue with statistical analysis to know the structure population of tuna in Indonesia archipelagic waters.

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