NIWA

Taihoro Nukurangi

Stock relationships of alfonsino and cardinalfish in New Zealand waters

Peter Smith, Ben Diggles, Brian Bull, Peter Benson

Final Research Report for
Ministry of Fisheries Research Project DEE1999/03
Objective 2

National Institute of Water and Atmospheric Research

September 2001

Final Research Report

Report Title: Stock relationships of alfonsino and cardinalfish in New Zealand waters

Authors: Peter Smith, Ben Diggles, Brian Bull, Peter Benson

1. **Date:** September 2001

2. **Contractor:** National Institute of Water and Atmospheric Research Limited

3. **Project Title:** Stock relationships of alfonsino and cardinalfish in New Zealand waters

4. **Project Code:** DEE1999/03

5. **Project Leader:** Peter Smith

6. **Duration of Project:**
   Start date: 1 October 1999
   Completion date: 30 September 2001

7. Executive Summary

Two stock discrimination methods, morphometrics and parasites, were evaluated in alfonsino from BYX 2 and BYX 3 and in cardinalfish from QMA 1 and QMA 2. Samples were collected opportunistically through NIWA voyages and shed sampling programmes. Preliminary morphometric analyses of alfonsino samples collected in July 2000 from BYX 2 and BYX 3 showed a significant difference between areas. Analysis of additional samples from BYX 2 and BYX 3 collected in November 2000 and January 2001 revealed both spatial and temporal variation. The temporal variation may be due to sub-area, seasonal, or reader differences in measurements. Future morphometric sampling should be undertaken on research vessels, or by observers on commercial vessels, to record location data and to collect specimens from a known number of tows. Samples should be taken from the same size range of fish (>30 cm) and in the same season. Spatial and temporal variation should be determined within one management area.

Two species of parasite, the cyst forming sporozoan, *Kudoa* sp., and the larval cestode, *Hepatoxylon trichiuri*, showed significant variation in prevalence and/or abundance in alfonsino from BYX 2 and BYX 3. *Kudoa* sp. was more prevalent in fish from BYX 2, while *H. trichiuri* was more abundant in BYX 3. Future parasite sampling should be based on specimens from known tows to test within and between area variation. It is recommend that sampling be based on 10 fish from 10 tows per area, and from the same size range of fish (>30 cm) in the same season. Samples should be frozen (or formalin fixed) at sea for preservation of parasites.

No morphometric differences were found between two samples of cardinalfish taken from QMA 1 and QMA 2. No further work with this tool is recommended for cardinalfish. Likewise no differences were found in parasite abundance and prevalence between samples from QMA 1 and QMA 2. However whole cardinalfish samples, taken when ice-vessels arrive in port are not ideal for parasite sampling. Any future parasite sampling should be based on specimens sampled at sea and from known tows (ideally 10 fish from 10 tows) to determine within and between area variation in parasite abundance and prevalence.

8. Objectives

Overall Objective

To determine stock relationships of alfonsino (*Beryx splendens*) and cardinalfish (*Epigonus telescopus*) within the New Zealand EEZ.

Specific Objectives

1. To determine the feasibility of discriminating stocks of alfonsino and cardinalfish within the New Zealand EEZ.

2. To determine the stock relationships for alfonsino in BYX 2 and BYX 3 and for cardinalfish in QMAs 1 and 2 if the feasibility study undertaken as part of Objective 1 proves successful.

9. Methods

Objective 2:

To determine the stock relationships for alfonsino in BYX 2 and BYX 3 and for cardinalfish in QMAs 1 and 2.

9.1 Background

The second objective was undertaken following a report on potential stock discrimination methods for alfonsino and cardinalfish (Smith and Paul 2000), and from feedback following a report presented to the Deepwater Fisheries Working Group (Greta Point, February 2000). The key conclusions were:

Alfonsino

- Alfonsino have a wide distribution in the Atlantic, Indian, and Pacific Oceans where they are most abundant between 300–500 m. The species appears to be subdivided into regional stocks contained within large-scale oceanic eddy systems. The larvae and juveniles are pelagic and drift considerable distances from the spawning area; adults do not appear to make extensive migrations.

- The New Zealand alfonsino fishery is centred on two areas off the Wairarapa coast (BYX 2), and to the east of the Chatham Islands (BYX 3). Spawning has not been recorded in New Zealand waters but large and maturing fish are caught off the Hawke Bay region, and larvae probably become trapped in the Wairarapa eddy system. It is not known if alfonsino also spawn off the Chatham Islands and if the larvae become entrapped in a separate eddy east of the Chatham Islands.

- Discrimination techniques that measure characteristics that are acquired (parasites) or determined (morphometrics) late in the life cycle are appropriate for a species with wide larval dispersal and limited adult movement.

Cardinalfish

- There is limited information on the biology and potential stock structure of cardinalfish. The species occurs in the Atlantic, Indian, and south west Pacific Oceans at depths of 200–1400m. The juveniles are pelagic, but adult movements are unknown. The species is widespread in the New Zealand EEZ, with many records from bycatch in the orange roughy fisheries. Two fisheries have developed: in the western Bay of Plenty (QMA 1) and on the Ritchie Hills outside Hawke Bay (QMA 2).

- Appropriate stock discrimination techniques would be based on characters/markers developed/acquired late in the cycle such as morphometrics and parasites.

The two different stock discrimination methods, morphometrics and parasites, have been evaluated in both alfonsino and cardinalfish.

9.2 Sample collection

Sample collection was undertaken opportunistically through NIWA research voyages and through commercial landings in Auckland, Gisborne, and Nelson to keep costs at a minimum. The first sets of samples, 50 alfonsino and 50 cardinalfish per management area, were collected in July and August 2000 (Table 1). Whole fish were sampled in processing sheds or at sea, where they were frozen whole or fixed in 10% formaldehyde. For alfonsino a second set of samples was collected in November 2000 and January 2001 (Table 1). Station location and tow data were not available other than for alfonsino samples collected as by-catch during an orange roughy survey on the Chatham Rise in July 2000 on the *FV San Waitaki* (SWA0001), and a hoki survey on the Chatham Rise in January 2001 on the *RV Tangaroa* (TAN0101).

Table 1: Collection details for alfonsino and cardinalfish used for morphometric and parasite analyses. * samples collected in shore-processing plant when vessel unloaded

Area	Region	Sample	Port/vessel	Company	Date
Alfonsino					
BYX 3	E Chatham Rise	Whole frozen	*San Waitaki*	Sanford	7.00
BYX 3	E Chatham Rise	Whole frozen	*Tangaroa*	NIWA	1.01
BYX 2	Wairarapa	Whole frozen & formaldehyde	Gisborne *	Moana Pacific	7.00
BYX 2	Wairarapa	Whole frozen	*Amaltal Mariner* *	Amaltal	11.00
Cardinalfish					
QMA 1	Bay of Plenty	Whole frozen	Auckland *	Anton Seafoods	7.00
QMA 2	Hawke Bay	Whole frozen & formaldehyde	Gisborne *	Gisborne Fisheries	8.00

9.3 Morphometric data collection

Landmarks were selected for each species based on characters in the published literature (Mayer 1974, Ivanin 1989) and on examination of specimens. External landmarks consisted of readily identifiable points on the margin of the fish, such as end of snout and insert of dorsal fin (Figures 1 & 2). Internal landmarks consisted of readily identifiable points on the body, such as pectoral fin and upper maxillary.

Sheets of waxy paper had been printed with an appropriate oblong to contain the largest specimen of each species (500 x 200 mm for alfonsino and 879 x 209 mm for cardinalfish). One sheet was used per specimen. Thawed specimens were blotted dry and placed on a wax sheet, within the oblong, and the position of the external landmarks marked on the sheet by placing a steel dissecting needle against each "external" landmark and piercing the wax paper. Internal landmarks were measured with calipers and the data recorded in an Excel spreadsheet.

The external landmarks on the marked sheets were digitised using Autocat Map 2000 software. The data were processed with ArcInfo and recorded as individual csv files and transferred to an Excel spreadsheet.

9. 3.1 Alfonsino morphometrics

Twelve external landmarks were selected around the alfonsino perimeter to give 17 morphometric measures as shown in Figure 1 and listed in Table 2. Four "internal" landmarks were selected as listed in Table 2. In addition, for each specimen the sex was recorded, the number of dorsal fin spines recorded, and a piece of white muscle tissue taken for future genetic analyses. The number of dorsal spines was the only meristic character to differentiate alfonsino stocks in the Indian Ocean (Ivanin 1989).

9.3.2 Cardinalfish morphometrics

Eleven external landmarks were selected around the cardinalfish perimeter to give 18 morphometric measures as shown in Figure 2 and listed in Table 3. Five "internal" landmarks were selected as listed in Table 3. In addition, for each specimen the sex was recorded and a piece of white muscle tissue taken for future genetic analyses.

9.4 Morphometric statistical analyses

Morphometric characters are related to fish size, therefore sub-samples covering the same length range were compared between areas.

All morphometric variables except standard length were converted into "anomalies" with respect to standard length, i.e., deviations from the typical value of the character among fish of that length in the combined samples. This was done to remove the confounding effect of fish size on the characters. The anomaly for each character was calculated as the residuals from a loess regression of the character on standard length. Loess regression was used to allow for non-linear relationships between characters and fish length (Venables & Ripley 1999).

Multivariate analysis of variance (MANOVA) was used to compare the two area samples on the basis of the anomalised characters. Fish sex was also included in the model as a predictor variable affecting character values. The MANOVA tests the combined significances of the between-sample differences in characters, allowing for the correlations between characters.

Mean between-sample differences were estimated for each anomalised character in the MANOVA analysis. The significance of each difference was assessed using a *t*-test, to indicate which characters contributed most to the overall difference between samples.

Table 2: Morphometric characters recorded in alfonsino specimens.

Code No	Landmarks	Character description
	External	
1	1–2	Standard length
2	1–8	Distance from snout to rear anal fin insert
3	1–9	Distance from snout to insertion of anal fin
4	1–10	Distance from snout to insertion of left pelvic fin
5	1–2	Distance from snout to head notch
6	1–3	Distance from snout to insertion of dorsal fin
7	3–4	Length of base of dorsal fin
8	3–10	Dorsal fin insert to pelvic fin insert
9	3–9	Dorsal fin insert to anal fin insert
10	4–5	Dorsal fin insert to dorsal caudal fin insert
11	4–10	Rear dorsal fin insert to pelvic fin insert
12	4–9	Rear dorsal fin insert to anal fin insert
13	4–8	Rear dorsal fin insert to rear anal fin insert
14	5–8	Rear anal fin insert to dorsal insert of caudal fin
15	5–7	dorsal to ventral insert of caudal fin
16	8–9	Length of base of anal fin
17	11–12	Mandible length
	Internal	
A1	3a	Distance from snout to operculum
A2	4a	Orbit diameter
A3	5a	Post orbit to operculum
A4	6a	Distance from snout to insertion of left pectoral fin

Table 3: Morphometric characters recorded in cardinalfish

Code No	Landmarks	Character description
1	1–2	Snout to first dorsal fin (D1)
2	2–3	Length D1
3	4–5	Length second dorsal fin (D2)
4	1–7	Standard length
5	6–8	Depth caudal peduncle
6	9–10	Length anal fin
7	6–9	Body depth
8	4–9	Body depth
9	5–9	Body depth
10	2–11	Body depth (D1-Pc)
11	2–10	Body depth (D1 –A)
12	4–11	Body depth (D2-Pc)
13	4–10	Body depth D2-A)
14	1–11	Snout to pelvic fin
15	1–10	Snout to anal fin
16	1–4	Snout to D2
17	5–6	D2 rear to caudal peduncle
18	5–8	Body depth
	Internal	
A1	1a	Snout to anterior orbit
A2	2a	Orbit diameter
A3	3a	Snout to operculum spine
A4	4a	Snout to maxilla
A5	5a	Distance from snout to insertion of left pectoral fin

9. 5 Parasite data collection

One hundred and twenty alfonsino and 50 cardinal fish were dissected and examined for parasites. Formalin fixed (n = 30) and frozen (n = 30) alfonsino from BYX 2, and frozen specimens (N = 60) from BYX 3 were examined. Frozen cardinalfish were examined from QMA 1 (n = 26) and QMA 2 (n = 24). Prior to dissection, frozen specimens were thawed overnight, while formalin fixed specimens were washed in water to remove excess fixative. Each thawed/washed specimen was measured to the nearest 0.5 cm (fork length) before parasites in the gills and guts were dissected under a dissecting microscope, and the types and numbers of parasites present recorded for each fish.

The ecological terminology used to describe the distribution of parasites amongst fishes followed that recommended by Bush et al. (1997):

- Prevalence = number of infected fish divided by number of fish examined,
- Intensity = number of parasites found in a sample of infected fish, and
- Abundance = number of parasites found in a sample of both infected and uninfected fish.

The criteria used to determine whether a parasite had potential for use as a stock discriminator followed those described by MacKenzie (1983, 1987) and Lester (1990), with emphasis on the following:

- the parasite should have a lifespan, or remain in identifiable form in the host, long enough to cover the time scale of the investigation (in this case, long lived parasites),
- the parasite should occur at a reasonably high prevalence, arbitrarily set for this study at >10% at one or more sites,
- the parasite should be easily detected and identified, and
- the method of examination should involve a minimum of dissection.

9.6 Parasite statistical analyses

Between-area differences in parasite abundance were tested using randomisation tests. Regression methods used in previous studies (Smith et al. 2000) were not used because sample sizes were relatively small. For each parasite, the null hypothesis was that there was no significant difference in parasite abundance between areas. The test statistic was the between-area difference in mean abundance. Since parasite abundances can depend on fish size, fish were grouped into 5 cm length classes of 30–34.9, 35–39.9, and 40–44.9 cm for alfonsino and 50–54.9, 55–59.9, and 60–64.9 cm for cardinalfish (Tables 4 and 5). Parasite data for fish which were smaller than these length ranges were discarded. A total of 500 bootstrapped datasets were generated for each species, and a *P*-value was calculated for each parasite by comparing the observed value of the test statistic with the 500 bootstrapped values. A *P* value of less than 0.05 was considered significant.

The above analysis does not consider within area variation in parasite abundance. The presence of between-tow variation within areas could lead to spurious between-area differences, when only 2–3 samples are available from each area. For alfonsino, but not cardinalfish, tow data were available, allowing a test of between-tow variation.

Table 4: Number and size range of alfonsino from BYX2 and BYX 3 dissected for parasites

Size range (mm)	BYX 2	BYX 3	All areas
< 299	2	23	25
300–349	6	6	12
350–399	31	13	44
400–449	21	18	39
Mean fish length (mm)	390	354	392
Total number of fish	60	60	120

Table 5: Number and size range of cardinalfish from QMA 1 and QMA 2 dissected for parasites

Size range (mm)	QMA 1	QMA 2	All areas
<499	0	4	4
500–549	3	8	11
550–599	11	6	17
600–649	12	6	18
Mean fish length (mm)	583	570	577
Total number of fish	26	24	50

10. Results

10.1 Morphometrics, alfonsino July 2000 samples

The size ranges of the alfonsino collected in July 2000, from BYX 2 and BYX 3, and used for morphometric analyses are given in Table 6. The overlap in size range in the two July samples was from 31-40 cm standard length, for which there were 50 fish from BYX 2 and 15 fish from BYX 3 (Table 6).

Table 6: Number and size range of alfonsino from two management areas, used for the July morphometric analyses

	Size (mm)							
Area	300–20	321–41	341–60	361–80	381–400	401–20	421–40	441–60
BYX 2	1	10	14	13	12			
BYX 3	1	2	3	5	4	4	13	3

An indication of the typical value of each character, the average value for a BYX 2 fish of a typical standard length of 36 cm is presented in Table 7. These averages are based on loess regressions of the characters on standard length. The MANOVA analysis showed a significant difference between samples ($P = 0.004$). The difference between sexes was not significant ($P = 0.07$). The results for individual characters are given in Table 7.

The number of dorsal spines showed no variation with 4 spines counted in each specimen.

The initial conclusion is that there are significant differences in alfonsino shape between BYX 2 and BYX 3, but these differences need to be tested in additional samples of fish.

10.2 Morphometrics, all alfonsino samples

The size ranges for all the alfonsino specimens used in the morphometric analyses are given in Table 8.

An indication of the typical value of each character, the average value for a BYX 2 fish of a typical standard length of 36 cm is presented in Table 9. These averages are based on loess regressions of the characters on standard length. The MANOVA analysis showed a significant difference between samples ($P < 0.0001$). The difference

between sexes was not significant ($P = 0.80$). The results for individual characters are given in Table 9.

The number of dorsal spines showed no variation with 4 spines counted in each specimen.

The combined results (Table 9) are quite different from those observed using only the July 2000 samples (Table 7). Some between-area differences which were observed in the original dataset are no longer present in the combined datasets, and some new differences have arisen. For the July data, six morphometric characters showed significant differences between areas (Table 7) and these differences cluster around the dorsal, anal, and caudal fins (5/6 characters). In the combined data, seven morphometric characters showed significant heterogeneity (Table 9) but only two characters (dorsal fin insert to dorsal caudal fin insert – line 10, Fig 1; and dorsal to ventral caudal fin insert – line 15, Fig 1) were common with the July differences. In the combined data 4/7 characters showing significant differences cluster around the head (Table 9).

Plots of character values for the same area, from the July and November/January samples, show between-sample differences. For example, fish of a given length from the November 2000 BYX 2 sample appear to have a greater snout to operculum length than fish of the same length from the July 2000 sample (Fig. 3). The same pattern of within area differences is seen for several other characters (snout to head notch, line 5; dorsal fin base length, line 7; mandible length, line 17). Therefore combining the July and November/January data sets is invalid.

In addition to length related differences in morphometric characters, there may be sub-area differences, seasonal differences, or reader error driving the within area differences. The initial results indicate significant morphometric differences between BYX 2 and BYX 3 in the winter (July); the additional samples (November/January) indicate a difference between sub-areas or between seasons. Sub-area differences could arise if there is limited movement of adults and there are different feeding conditions/growth rates among sub-areas. Alternatively temporal differences in shape might reflect seasonal spawning or feeding conditions. However the key characters showing temporal differences are clustered around head shape and are least likely to reflect seasonal changes in diet/reproductive state, which would be expected to influence abdominal cavity shape. The same reader was used to undertake all morphometric measurements, but the data were collected at two time periods and could therefore reflect reader error.

Table 7: Between-sample differences (mm) for each anomalised morphometric character in alfonsino as BYX 2 minus BYX 3. *P*-values are calculated by the standard *t*-test. Typical values of the character for a 36 cm alfonsino from BYX 2 are given. * significant at the 5% level ** significant at 1% level

Character	Between-sample difference		*P*-value	Typical value
Snout to operc. Length	-0.1		0.87	110.7
Orbit diameter	0.1		0.77	49.4
Orbit to operc. Length	-0.7		0.28	42.8
Snout to ins. Left pect. Fin	0.8		0.88	108.7
Dorsal fin ins. To dorsal caudal fin ins.	2.5	(*)	0.013	189.2
Rear dorsal fin ins. to pelvic fin ins.	-0.3		0.96	149.3
Rear dorsal fin ins. to anal fin ins.	3.0	(**)	0.005	116.6
Rear dorsal fin ins. to rear anal fin	2.5	(**)	0.004	105.6
Rear anal fin ins. to dorsal ins. caudal fin	1.5	(*)	0.05	52.1
Dorsal to ventral ins. caudal fin	1.5	(**)	0.0004	39.8
Base anal fin length	-1.0		0.50	103.6
Mandible length	-1.5		0.10	73.5
Snout to rear anal fin ins.	0.8		0.31	302.9
Snout to anal fin ins.	-0.4		0.66	223.5
Snout to left pelvic fin ins.	-0.4		0.57	156.4
Snout to head notch	0.3		0.80	47.1
Snout to dorsal fin ins.	3.1	(**)	0.008	158.0
Dorsal fin base length	0.4		0.44	73.7
Dorsal fin ins. To pelvic fin ins.	-0.4		0.88	136.0
Dorsal fin ins. To anal fin ins.	1.6		0.16	144.0

Table 8: Number, size range, and date of collection of alfonsino from BYX 2 and BYX 3, used for morphometric analyses

	Area and date (month/year) of collection			
Size range	**BYX 2**	**BYX 3**	**BYX 2**	**BYX 3**
(mm)	**7.00**	**7.00**	**11.00**	**1.01**
221–240				1
241–260				3
261–280				3
281–300				3
301–320	1	1		0
321–340	10	2		1
341–360	14	3		3
361–380	13	5	2	1
381–400	12	4	18	
401–420		4	21	
421–440		13	7	
441–460		3	4	

Table 9: Between-sample differences (mm) for each anomalised morphometric character in alfonsino as BYX 2 minus BYX 3, based on all samples. *P*-values are calculated by the standard *t*-test. Typical values of the character for a 36 cm alfonsino from BYX 2 are given. * significant at the 5% level ** significant at the 1% level

Character	Between-sample difference		*P*-value	Typical value
Snout to operc. Length	3.7	(**)	<0.001	110.9
Orbit diameter	-0.2		0.67	49.3
Orbit to operc. Length	-0.4		0.43	43.0
Snout to ins. Left pect. Fin	4.8	(**)	<0.001	113.9
Dorsal fin ins. To dorsal caudal fin ins.	2.3	(**)	0.004	189.0
Rear dorsal fin ins. to pelvic fin ins.	-1.6		0.35	149.8
Rear dorsal fin ins. to anal fin ins.	0.9		0.14	116.8
Rear dorsal fin ins. to rear anal fin	1.2		0.05	105.4
Rear anal fin ins. to dorsal ins. caudal fin	-0.4		0.39	52.0
Dorsal to ventral ins. Caudal fin	0.9	(**)	0.001	40.0
Base anal fin length	0.9		0.16	103.3
Mandible length	-1.7	(**)	0.005	73.8
Snout to rear anal fin ins.	1.1		0.05	302.7
Snout to anal fin ins.	-0.4		0.66	224.4
Snout to left pelvic fin ins.	-1.2		0.12	157.1
Snout to head notch	1.4	(*)	0.03	47.4
Snout to dorsal fin ins.	0.6		0.40	157.4
Dorsal fin base length	0.5		0.40	73.4
Dorsal fin ins. To pelvic fin ins.	-2.5	(*)	0.05	136.4
Dorsal fin ins. To anal fin ins.	0.1		0.85	144.2

10.3 Morphometrics, cardinalfish

The overlapping sizes of the cardinalfish ranged from 48-62 cm standard length, for which there were 49 fish from QMA 1 and 44 fish from QMA 2 (Table 10). An indication of the typical value of each character, the average value for a QMA 2 fish of a typical standard length of 55 cm is presented in Table 11. These averages are based on loess regressions of the characters on standard length.

Table 10: Number and size range of cardinalfish from the two management areas, used for morphometric analyses

	Size (mm)											
Area	440–60	461–80	481–00	501–20	520–40	541–60	561–80	581–00	601–20	621–40	641–60	661–80
QMA 1	1	2	6	7	9	4	7	7	4	1	2	
QMA 2			1	3	6	11	14	7	7			1

The MANOVA analysis showed no significant difference between samples ($P = 0.09$). The difference between sexes was not significant ($P = 0.21$). The results for individual characters are given in Table 11. There were significant results at the 5% level for the snout to first dorsal (Fig. 2, landmarks 1–2) and body depth (Fig. 2, dorsal 1-anal fin; landmarks 2–10), however the MANOVA result indicated that these differences are no more than might be expected to occur by chance when this many characters are tested.

10.4.1 Alfonsino parasites

Parasites found in alfonsino included two species of cyst forming sporozoans (*Kudoa* sp. and a microsporidian) on the gills and gill arches; a monogenean worm (*Microcotyle* sp.) and a copepod (*Neobrachiella* sp.) in the gills; one unidentified species of larval cestode in the mesentries and another (*Hepatoxylon trichiuri*) in the body cavity; larval nematodes in the body cavity and mesentries (*Anisakis* type 1, 2 and 4 larvae); and one species of adult nematode (*Hysterothylacium* sp.) and one species of digenean worm inside the stomach (Table 12). Of these species, the cyst forming sporozoans, the larval cestodes and the larval nematodes had biological characteristics which made them potentially informative parasites. Of these, the microsporidian and the unidentified species of larval cestode both had a very low prevalence (0.8% overall and 4.2% overall, respectively), and only the larval nematode, *Kudoa* sp. and the remaining larval cestode *Hepatoxylon trichiuri* fulfilled all the criteria for potentially informative parasites.

Of the potentially informative parasites of alfonsino, *Kudoa* sp. was significantly more prevalent ($P = 0.02$) in BYX 2 where it was found in 28.3% of fish, compared to only 5% of fish in BYX 3. The prevalence and intensity of live *Hepatoxylon trichiuri* larvae in fish sampled from BYX 3 was significantly higher ($P < 0.01$) than in fish from BYX 2 (Table 13). The abundances of dead and pooled alive and dead *H. trichiuri* were also significantly higher ($P < 0.01$) in fish from BYX 3 than BYX 2.

When the data for *H. trichiuri* (pooled dead and alive) infections in different size classes of alfonsino were analysed for different trawl tows or trawl periods, within area variation in fish size and mean *H. trichiuri* abundance was evident (Table 14). However most cell N's are low, typically 5 or less (Table 14), and these are too low to use re-sampling or regression methods to assess between-tow/period differences in the presence of variation due to length.

Table 11: Between-sample differences (mm) for each anomalised morphometric character in cardinalfish, as QMA 2 minus QMA 1. *P*-values are calculated by the standard *t*-test. Typical values of the character for a 55 cm fish from QMA 2 are given. * significant at the 5% level

Character (landmarks)	Between-sample difference	*P*-value	Typical value
snout to anterior orbit	1.0	0.08	48.1
orbit diameter	-0.5	0.48	55.3
snout to operc. Spine	1.9	0.12	176.6
snout to ins. Left pect.(pc) fin	2.6	0.07	187.8
Maxilla length	0.5	0.37	79.4
snout to dorsal (d)1 (1–2)	-2.2	(*) 0.02	209.0
Body depth (d1–pc) (2–11)	1.4	0.46	146.0
Body depth (d1–anal) (2–10)	2.6	(*) 0.02	205.7
Body depth (d2–pc) (4–11)	-0.8	0.45	201.5
Body depth (d2–a) (4–10)	2.0	0.09	125.2
snout to pelvic fin (1–11)	1.4	0.32	194.8
snout to anal fin (1–10)	-0.1	0.83	374.8
snout to d2 (1–4)	-2.0	0.08	323.6
d2rear to caudal ped (5–6)	1.5	0.29	125.6
Body depth (5–8)	1.0	0.46	143.5
length d1 (2–3)	1.4	0.27	66.4
length d2 (4–5)	0.0	0.96	54.1
depth (6–8) caudal ped	0.2	0.87	48.3
length anal fin (9–10)	0.2	0.81	44.4
Body depth (6–9)	0.6	0.66	107.9
Body depth (4–9)	1.0	0.33	134.7
Body depth (5–9)	1.0	0.36	95.7

Table 12: Prevalence and mean abundance of parasites from alfonsino from BYX 2 and BYX 3

	Parasite prevalence (%)			Parasite mean abundance		
	BYX 2	**BYX 3**	**All areas**	**BYX 2**	**BYX 3**	**All areas**
Sporozoa						
Kudoa sp.	28.3	5	16.7	1.3	0.18	0.74
Microsporidian	1.7	0	0.8	0.03	0	0.02
Monogenea						
Microcotyle sp.	18.3	11.7	15	3.5	0.73	2.1
Nematoda						
Anisakis type 1 larvae	30	35	32.5	0.43	0.63	0.53
Anisakis type 2 larvae	13.3	3.3	8.3	0.15	0.03	0.09
Anisakis type 4 larvae	11.7	1.7	6.7	0.15	0.02	0.08
Anisakis sp. pooled	41.7	40	40.8	0.73	0.68	0.71
Hysterothylacium sp.	28.3	13.3	20.8	0.52	0.45	0.48
Cestoda						
Liver plerocercoid	8.3	0	4.2	0.13	0	0.07
Hepatoxylon trichiuri	11.7	26.7	19.2	0.15	0.47	0.31
Hepatoxylon trichiuri (dead)	90	76.7	83.3	3.3	4.2	3.8
Hepatoxylon trichiuri (pooled alive and dead)	90	78.3	84.2	3.5	4.7	4.1
Crustacea						
Neobrachiella sp.	1.7	3.3	2.5	0.02	0.05	0.03

Table 13: Results of randomisation tests for differences in the prevalence and abundance of parasites of alfonsino from areas BYX 2 and BYX 3. * significant difference at the $P < 0.05$ level, ** significant difference at the $P < 0.01$ level

Parasites from alfonsino	*P*-value for between-area differences in prevalence	*P*-value for between-area differences in abundance
Kudoa sp.	(*) 0.02	0.07
Microsporidian	0.27	0.27
Microcotyle sp.	0.81	0.56
Anisakis type 1 larvae	0.25	0.33
Anisakis type 2 larvae	0.15	0.15
Anisakis type 4 larvae	0.06	0.09
Anisakis pooled	0.12	0.09
Hysterothylacium sp.	0.21	0.11
Cestode larvae	0.08	(*) 0.03
Hepatoxylon trichiuri	(**) <0.01	(**) <0.01
Dead *Hepatoxylon trichiuri*	0.48	(**) <0.01
Alive and dead *H. trichiuri*	0.51	(**) <0.01
Neobrachiella sp.	0.51	0.51

Table 14: Mean abundance of *Hepatoxylon trichiuri* (pooled dead and alive) for three length classes of alfonsino in three trawl tows/periods from BYX 2 and four trawl tows from BYX 3

Area	Tow	Date	Mean abundance of *Hepatoxylon trichiuri* by length class		
			300–349 mm	350–399 mm	400–449 mm
BYX 2	1	7.00	3.6 (n=5)	2.6 (n=18)	1.9 (n=7)
	2	7.00	7.0 (n=1)	6.1 (n=10)	4.3 (n=3)
	3	11.00		1.3 (n=3)	3.8 (n=11)
BYX 3	1	7.00	9.0 (n=1)	13.0 (n=6)	3.5 (n=12)
	2	7.00	5.0 (n=1)		7.5 (n=2)
	3	7.00	4.0 (n=1)	12.5 (n=2)	5.0 (n=4)
	4	1.01	8.7 (n=3)	2.4 (n=5)	

10.4.2 Cardinalfish parasites

Cardinalfish had a lower parasite species diversity than alfonsino. However both frozen and formalin fixed specimens were in poor condition. The fish had been held on ice for a few days prior to processing, resulting in autolysis of the internal organs, which made it difficult to locate and identify parasites in the body cavity and gut.

The cardinalfish from QMA 1 had two species of monogenean worm on the gills (*Diclidophora* sp. and *Winkenthughesia* sp.), an adult nematode (*Hysterothylacium* sp.), an adult acanthocephalan and one species of digenean worm in the stomach, and larval nematodes (*Anisakis* type 1) and cestodes encysted in the mesentries (Table 15). The cardinalfish from QMA 2 did not have *Winkenthughesia* sp., nor *Hysterothylacium* sp. or the digenean in the stomach (Table 15). Both the larval cestode and larval nematode have biological characteristics which make them potentially informative parasites, however due to the low prevalence of the larval cestode (4% overall), only the larval nematode *Anisakis* sp. fulfilled all criteria required of a potentially informative parasite. The remaining parasites were considered likely to be short lived and were not considered to have potential as useful long term population markers. The data on *Anisakis* sp. larvae, displayed no

significant between area differences in either prevalence (P = 0.09) or abundance (P = 0.88). One species, the relatively short lived nematode *Hysterothylacium* sp., showed significant variation between QMA 1 and QMA 2 in prevalence and abundance (Table 16).

No further sampling of cardinalfish was undertaken for parasite studies due to (i) the lack of between area variation in *Anisakis* and lack of other suitable parasite markers found in this preliminary analysis; (ii) the poor condition of cardinalfish derived from the fishery; and (iii) the lack of tow data from the fishery samples.

Table 15: Prevalence and mean abundance of parasites from cardinalfish from QMA 1 and QMA 2

	Parasite prevalence (%)			Parasite mean abundance		
	QMA 1	QMA 2	All areas	QMA 1	QMA 2	All areas
Monogenea						
Diclidophora sp.	11.5	25	18	0.12	0.42	0.26
Winkenthughesia sp.	3.8	0	2	0.08	0	0.04
Nematoda						
Anisakis type 1 larvae	11.5	25	18	0.69	0.63	0.66
Hysterothylacium sp.	15.4	0	8	0.19	0	0.1
Cestoda						
Cestode larvae	3.8	4.2	4	0.04	0.08	0.06
Digenea						
Digenean 1	7.7	0	4	0.08	0	0.04
Acanthocephala						
Acanthocephalan	3.8	0	2	0.4	0	0.02

Table 16: Results of randomisation tests for differences in the prevalence and abundance of parasites of cardinalfish from areas QMA 1 and QMA 2. ** significant difference at the $P < 0.01$ level

Parasite	P-value for between-area difference in prevalence	P-value for between-area difference in abundance
Diclidophora sp.	0.40	0.15
Winkenthughesia sp.	0.34	0.34
Anisakis type 1 larvae	0.09	0.88
Hysterothylacium sp.	(**) <0.01	(**) <0.01
Cestode larvae	0.45	0.45
Digenean 1	0.10	0.10
Acanthocephalan	0.33	0.33

11. Conclusions

Alfonsino

1. Preliminary morphometric analyses of July (2000) samples from BYX 2 and BYX 3 showed a significant difference between areas. Additional sampling in November 2000 and January 2001 revealed both spatial and temporal variation. This temporal variation may be due to sub-area, seasonal, or reader differences in measurements.

2. Future morphometric sampling should be undertaken on research vessels, or by observers on commercial vessels, to record location data and to collect specimens from a known number of tows. Area samples should be taken from the same size range of fish (>30 cm) and in the same season. Spatial (=sub-area) and temporal variation should be determined within one management area.

3. Two species of parasite, the cyst forming sporozoan, *Kudoa* sp., and the larval cestode, *Hepatoxylon trichiuri*, showed significant variation in prevalence and/or abundance between BYX 2 and BYX 3. *Kudoa* sp. was significantly more prevalent in fish from BYX 2, while *H. trichiuri* was significantly more abundant in BYX 3. The limited tow/period data suggested within area variation in abundance of *H. trichiuri*, but the data were insufficient to statistically test this hypothesis.

4. Future parasite sampling should be based on specimens from known tows to test within and between area variation. We recommend sampling 10 fish from 10 tows per area. The samples should be collected from the same size range of fish (>30 cm) in the same season. Samples should be frozen (or formalin fixed) at sea for preservation of parasites.

Cardinalfish

1. No morphometric differences were found between two samples of cardinalfish taken from QMA 1 and QMA 2. No further work with this tool is recommended for cardinalfish.

2. No differences in parasite abundance and prevalence were found between samples from QMA 1 and QMA 2. However whole cardinalfish samples, taken when ice-vessels arrive in port are not ideal for parasite sampling. Any future parasite sampling should be based on specimens sampled at sea and from known tows (ideally 10 fish from 10 tows) to determine within and between area variation in parasite abundance and prevalence.

12. Publications

None.

13. Data Storage

Morphometric and parasite data are stored on the H: drive NIWA Greta Point.

REFERENCES

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W. 1997: Parasitology meets ecology on its own terms: Margolis *et al.* Revisited. *Journal of Parasitology 83*: 575–583.

Ivanin, N.A. 1989: Morphometric characters of the alfonsino, *Beryx splendens*, of the submarine ridges in the temperate zone of the Indian Ocean. *Voprosy Ikhtiologii 1*: 160–163.

Lester, R.J.G. 1990: Reappraisal of the use of parasites for fish stock identification. *Australian Journal of Marine and freshwater Research 41*: 855–864.

Lester R.J.G., Sewell K.B., Barnes A., Evans K. 1988: Stock discrimination of orange roughy, *Hoplostethus atlanticus*, by parasite analysis. *Marine Biology 99*: 137–143.

MacKenzie, K. 1983: Parasites as biological tags in fish population studies. In: Coaker, TH (ed) Advances in Applied Biology Vol. VII, Academic Press London, p. 251–331.

MacKenzie, K. 1987: Parasites as indicators of host populations. In: M. J. Howell (ed), Parasitology – Quo Vadit ? Proceedings of the 6th International Congress of Parasitology, Australian Academy of Science, Canberra. p. 345–352.

Mayer, G.F. 1974: A revision of the cardinalfish genus *Epigonus* (Perciformes, Apogonidae), with descriptions of two new species. *Bulletin Museum Comparative Zoology 146*: 147–203.

Smith J.W., Wootten, R. 1978: *Anisakis* and anisakiasis. *Advances in Parasitology 16*: 93–163.

Smith, P., McMillan, P., Proctor, C., Robertson, S., Knuckey, I., Diggles, B., Bull, B. 2000: Stock relationships of black oreo in New Zealand waters. Final Research Report to Ministry of Fisheries, November 2000.

Smith, P.J., Paul, L.J. 2000: Stock relationships of alfonsino and cardinalfish in New Zealand waters. Final Research Report to the Ministry of Fisheries August 2000.

Venables, W.N., Ripley, B.D 1999: Modern applied statistics with S-PLUS (3rd ed.). Springer-Verlag, New York. 501 p.

Fig 1. Perimeter landmarks and the 17 morphometric measures selected in alfonsino.

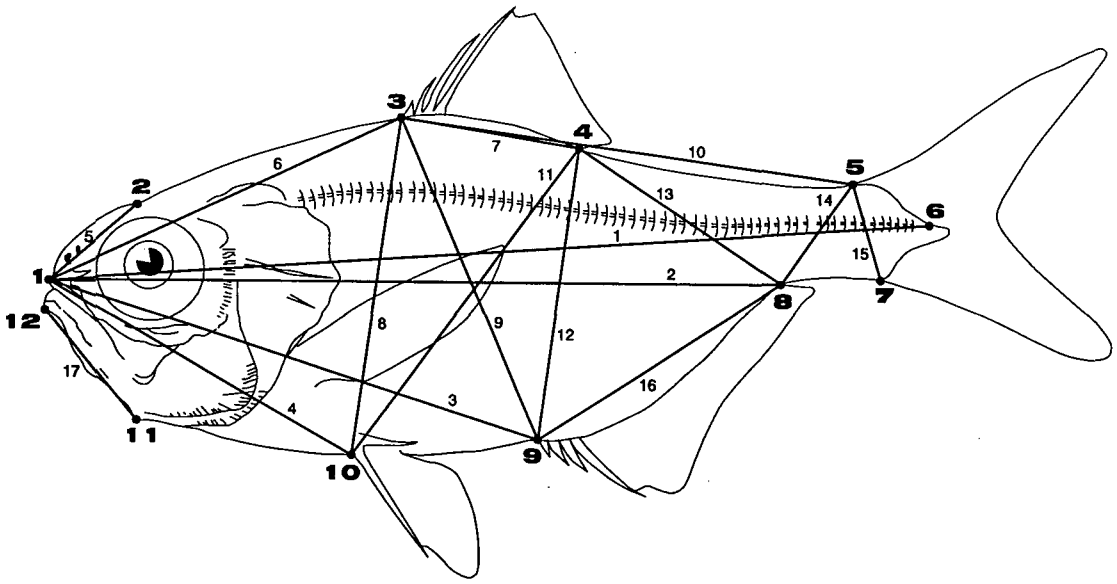

Fig. 2. Perimeter landmarks and the 18 morphometric measures selected in cardinalfish.

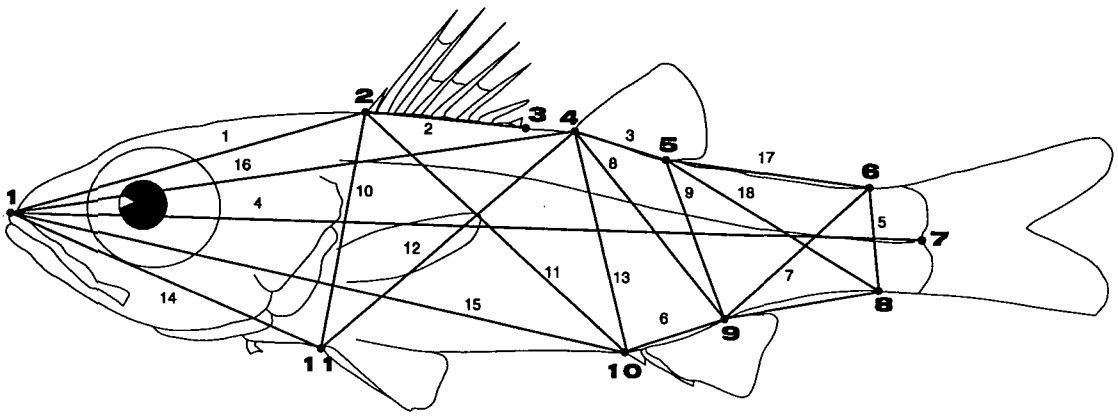

Fig. 3. Comparison of July (X) and November (O) 2000 alfonsino morphometric samples from BYX 2 for character snout to operculum/length.