Banggai cardinalfish (*Pterapogon kauderni*) populations (stocks) around Banggai Island, a geometric and classical morphometric approach

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ABSTRACT

Background. The identification and characterisation of appropriate management units (stocks) is important as a basis for responsible fisheries management as well as conservation of within species biodiversity. The Banggai cardinalfish *Pterapogon kauderni* (F.P. Koumans,1933), a mouthbrooding apogonid with Endangered status (IUCN Red List) has been shown to have a high level of genetic population structure across the endemic distribution in the Banggai Archipelago. With a life-cycle making recovery frrm extirpation extremely unlikely, this indicates a need to conserve each reproductively isolated population (stock), in particular to support zonation of Banggai Island in the context of the proposed district marine protected area. Genetic and morphological variations are often but not always related, and ideally both should be used in stock identification. However there were no data on classical or geometric morphometric characteristics of *P. kauderni* populations.

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Methods. Adult *P. kauderni* for classical and geometric morphometric analyses were collected randomly at six sites on Banggai Island (31-34 adult fish/site, total 193). Eleven morphometric parameters were measured and 10 dimensionless ratios were compared using the ANOVA function in Microsoft Excel 2007. A landmark set for *P. kauderni* was developed. Each specimen was photographed, digitised (tps.dig and tps.util). Characteristics of the six populations were analysed using Canonical Variate Analysis (CVA) and Discriminant Function Analysis (DFA) in MorphoJ geometric morphometric software to identify significant between-site variation. The results were compared with genetic, geophysical, bio-ecological and socio-economic data to determine meaningful stocks or management units.

Results. Except for one site pair (Monsongan and Tinakin Laut) we found significant or highly significant differences between sites (sub-populations) in morphometric characteristics, as well as from the CVA and DFA results. The greatest morphometric difference was between sub-populations at the north (Popisi) and southeast (Matanga) extremities of the Banggai Island *P. kauderni* distribution. The Popisi population was characterised by short/high head shape, Matanga by a more hydrodynamic shape (elongated with a more pointed head). These findings were consonant with genetic study results. We propose a population model with four closed populations and one metapopulation resulting in five *P. kauderni* stocks around Banggai Island.

Discussion. The observed pattern of morphometric variation could be related to geographical spread (radiation or North-South gradient), habitat-driven selection or growth patterns, stochastic events, or a combination. Such fine-scale sub-population or stock characterisation calls for intra-species conservation, with implications for the management of this restricted range endemic ornamental fish not only around Banggai Island but throughout the *P. kauderni* endemic distribution.

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INTRODUCTION

It is generally accepted that species management, including conservation measures and sustainable use, should be based on biologically and ecologically meaningful units or sub-populations, which for fishes are generally referred to as stocks. Beg and Waldman (1999) found that the term stock is somewhat ambiguous, concluding that stock definition should evolve with management requirements and technological advances, and advocated a holistic approach to fish stock identification based on morphometric, life history and genetic data. Reiss *et al*.(2009) stated that "an essential prerequisite of sustainable fisheries management is the matching of biologically relevant processes and management action", with fish stocks as the fundamental unit, but found that fish biology and management action are commonly mismatched. Waldman (2005) listed the following characteristics shared by a given stock: a physical life-cycle circuit; set of demographic influences; isolation allowing fine-tuning of specific morphological and genetic characteristics; and subjection to unnatural influences such as fishing pressure and pollution.

Kritzer and Sale (2004) writing on metapopulation ecology in the sea described three population structures: (A) closed local populations, with no ecologically meaningful exchange of individuals, highly localized dispersal distances; (B) a metapopulation or network of partially closed populations with nontrivial supply from other populations; and (C) a patchy population, within which individuals are distributed among discrete groups and local populations essentially draw from a common larval pool. For a given species these structures could be nested at different scales or different structures could occur in different environmental conditions, and the structure will greatly affect the impacts of conservation measures such as marine protected areas and other fisheries management tools. It logically follows that in the case of closed populations (stocks) with no meaningful exchange (gene flow) between them, protection of one of these will have no impact on the status of the others. Furthermore, if any one should become depleted, replenishment would be very unlikely and local extinction(s) would most likely be permanent, would involve the loss of any specific evolved traits and potentially of unique genetic strains and adaptations, thus reducing biodiversity at an intra-species (genetic and possibly phenotypic) level. From a within species biodiversity conservation viewpoint, Rocha *et al*., (2007) note that the front line in marine conservation genetics is the identification of management units, that phylogeography can assist in revealing isolated and unique lineages, and stress the importance of adequate protection for each of these reproductively isolated populations or stocks. Ward (2006) pointed out the importance of identifying population structure in the context of re-stocking or stock enhancement, in order to maintain population genetic characteristics through appropriate broodstock selection.

As Hammer and Zimmermann (2005) pointed out, it has become evident that genetic methods have great potential for population studies, however in stock identification they considered it important that other identifiers such as specific ecology, meristic or morphological traits should be congruent with genetic results, echoing the concern of Coyle (1998) that several methods should be used in identifying fish stocks. Genetic and morphological variations are often but not always related (Rocha *et al.*, 2007). While a literature search readily reveals growing numbers of genetic stock and population structure studies, there have been relatively few morphometric studies aimed at fish stock identification, most of which have concentrated on freshwater fishes (e.g. Adams *et al.*, 2007, Hossein *et al.*, 2010), or commercially important food fishery species with wide distributions (e.g. Turan, 2004), while studies where the results of genetic and morphometric analyses are compared such as Cabral *et al.* (2003), Vasconcellos *et al*. (2008) or Turan and Yaglioglu (2010) are still rare.

The Banggai cardinalfish *Pterapogon kauderni* (Koumans, 1933) is a marine fish of conservation concern for which such concerns are particularly relevant. A paternal mouthbrooding apogonid with direct development (Vagelli, 1999), the life history does not include a pelagic phase. Despite a tendency to ontogenetic shift in microhabitat (Vagelli, 2004; Ndobe *et al.*, 2008), the Banggai cardinalfish exhibits extreme philopatry (Kolm *et al.*, 2005). The species has been shown to have a high level of genetic population structure across the endemic distribution in the Banggai Archipelago, Central Sulawesi, Indonesia, with genetically distinct sub-populations (arguably stocks) occurring on the same island as little as 2-5km apart (Bernardi and Vagelli, 2004; Hoffman *et al.*, 2005; Vagelli *et al.*, 2009).

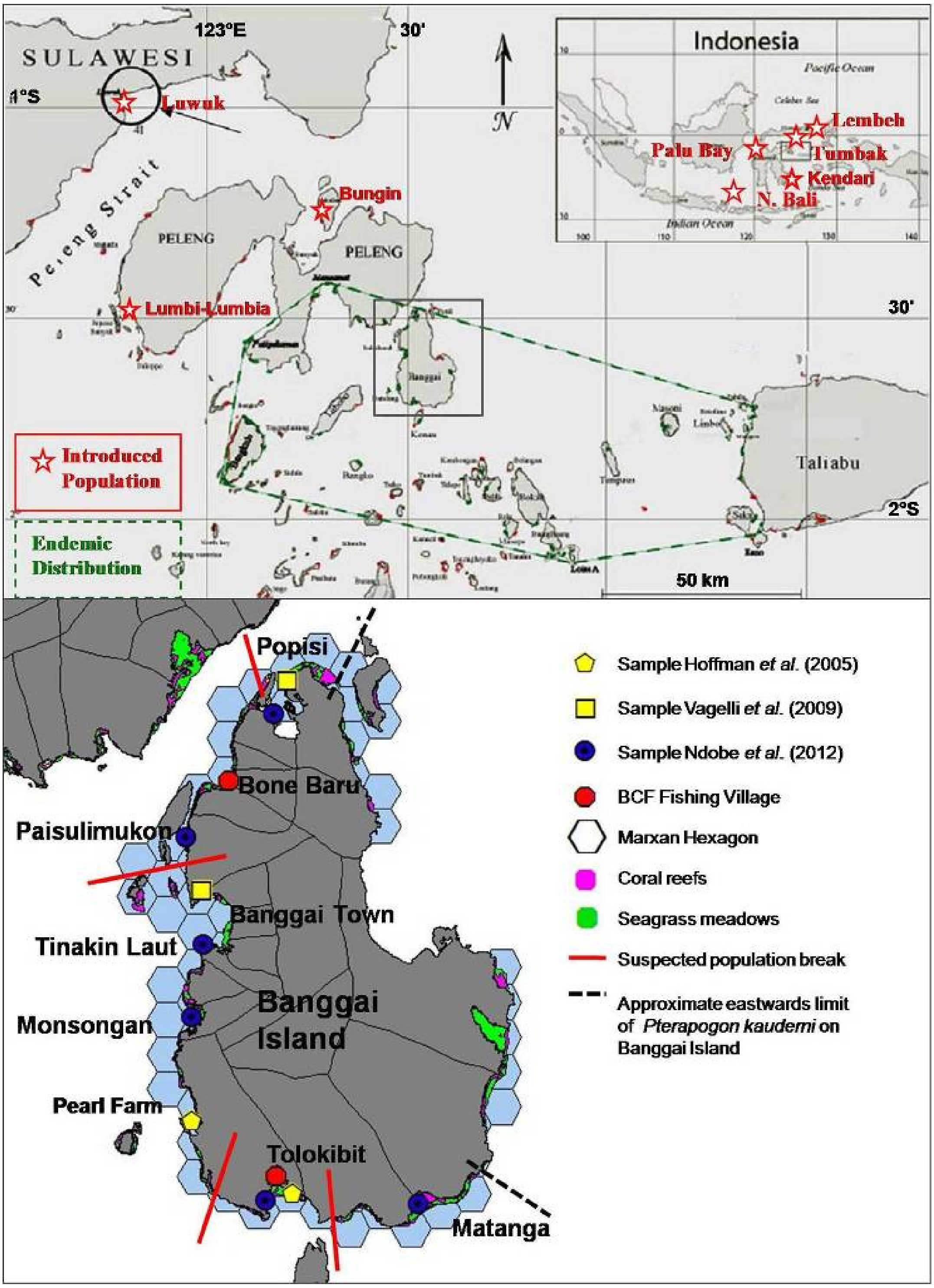
Traded as a marine ornamental in large numbers since the 1990's (Allen, 2000; Kolm and Berglund, 2003; Lunn and Moreau, 2004; Moore *et al.*, 2011), the conservation status of *P. kauderni* has become a national and international issue. Proposed for CITES listing in 2007 (Moore and Ndobe, 2007a; Indrawan and Suseno, 2008; Vagelli, 2008), *P. kauderni* was listed with Endangered status in the IUCN Red List later in the same year (Allen and Donaldson, 2007). The CITES proposal was withdrawn and Indonesia made a commitment to Banggai cardinalfish conservation with a sustainable ornamental fishery approach. At the District level the District head issued two decrees, establishing a Banggai Cardinalfish Centre (BCFC) and a marine protected area network consisting of ten islands, two of which were specifically designated for *P. kauderni* conservation. In fact, only one of these, Banggai

Island, actually has a *P. kauderni* population; Togong Lantang Island has a large *Sphaeramia nematoptera* population living among *Rhizophora* prop roots, which could have been mistakenly identified by inexperienced observers, but no Banggai cardinalfish (Ndobe *et al.*, 2012).

Banggai Island is the second largest island in the Banggai Archipelago, with an area of around 294km^2, over 37,000 inhabitants, 4 sub-districts. Of the 27 villages, at least 5 were involved in the Banggai cardinalfish ornamental fishery in 2004, and two villages are still active in this fishery: Bone Baru in the north of the island, arguably the most active centre of the Banggai cardinalfish trade in the archipelago, and Tolokibit in the south (Moore *et al.*, 2011). Bone Baru has been the focus of several government and NGO programs and has a community marine protected area (MPA), coral reef and mangrove conservation groups and an officially recognised ornamental fishers group. *Pterapogon kauderni* populations are distributed around the northern, western and southern coasts of the island, mostly in relatively protected bays or straits, but are not found on the more exposed eastern coast, and all are fished though with varying degrees of intensity.

Banggai cardinalfish habitat in the endemic distribution within the Banggai Archipelago is limited to shallow coastal waters with a maximum depth of 5-6m, including coral reefs, reef flats, seagrass beds, lagoons and at a few sites *Rhizophora* sp. prop roots (Vagelli and Erdmann, 2002; Moore *et al.*, 2012). Local extinction or extirpation (as defined by Woodruff, 2001) has been observed, with no recolonisation from populations a few hundred meters away but separated by deeper water (Ndobe *et al*., 2013). Around Banggai Island the distribution of *P. kauderni* populations appears to be discontinuous, with no fish observed or reported by fishermen along steeply sloping or more exposed coasts.

Combining the geophysical characteristics of the island with the life history traits of *P. kauderni*, it could be expected that the population structure of *P. kauderni* around Banggai Island would comprise several reproductively isolated or closed populations (Kritzer and Sale type A) and or one or more metapopulations (Kritzer and Sale type B) with limited connectivity, each of which should be considered as a separate management unit or stock. Indeed Hoffman *et al*. (2005) and Vagelli *et al*. (2009) each reported two sites (*P. kauderni* populations) with significantly different genetic characteristics. The genetic study approach presented in Ndobe *et al*. (2012) provided further information on 6 sites (Bone Baru where many fish from all over the endemic distribution had been released was purposely excluded), using the same pair of microsatellites (Pka06 and Pka11, Hoffmann *et al*., 2004) as Vagelli *et al*., (2009). The results indicated that there were at least 4 closed populations and one metapopulation, hence from a fisheries management and intra-specific conservation point of view, at least 5 stocks. The endemic distribution of *P. kauderni* (based on Vagelli, 2008), the sampling sites of all three studies and the suspected breaks due to geophysical characteristics (based on Ndobe *et al.*, 2012) as well as known introduced populations are shown in Fig. 1.



Sources: Erdman and Vagelli (2001); Vagelli and Erdmann (2002); Moore and Ndobe (2007b); Lilley (2008); Vagelli (2008); Moore *et al*. (2011); Ndobe *et al*. (2012); Ndobe and Moore (unpublished data)

Figure 1. *P. kauderni* endemic distribution, known introduced populations, suspected barriers to dispersion, genetic and morphometric sampling sites around Banggai Island

Traders had expressed preference for fish from certain sites, saying that the colours were brighter and more attractive, suggesting there might be external differences between populations and which could be due to genetic, environmental or other factors. Morphometric studies on *P. kauderni* concluded that there was no significant difference in external morphology between male and female Banggai cardinalfish (Vagelli and Volpedo, 2004; .Ndobe *et al*., 2013). However no morphometric studies had been published on *P. kauderni* populations. Furthermore, the relatively recent geometric morphometric approach is widely considered to have greater powers of resolution at an intra-species level than classical morphometrics (Slice, 2007; Madderbacher *et al*., 2008; Kerschbaumer and Sturmbauer, 2011; Klingenberg, 2011) but had not previously been applied to *P. kauderni*.

In this context it was considered important to study and compare the putative *P. kauderni* stocks around Banggai Island from a morphometric point of view, using classical as well as geometric morphometric methods and to compare the results with genetic and geophysical data, while taking into consideration known fishery/trading history. The results would provide information of use in both management of the ornamental fishery and the process of MPA planning, including zonation.

METHODS

Populations and Stocks

For the purposes of this study, the term population was considered to refer to the Banggai cardinalfish *P. kauderni* living within a particular geographic area. For example the Palu Bay (introduced) population, the endemic population in the Banggai Archipelago or the Popisi population. A given population should be considered to be a stock if it could be shown to have the characteristics listed by Waldman (2005), and or could be considered a closed population or metapopulation in the sense of Kritzer and Sale (2004).

Statistically significant variation in morphometric traits between populations could indicate genetic (reproductive) isolation and consequent divergent evolution due to factors such as the local environment, anthropogenic impacts and stochastic events (separate stocks). Such variation could also reflect adaptation to prevailing conditions at the individual level without necessarily defining separate stocks. Based on the precautionary principle, arguably management should aim to conserve each population exhibiting characteristics indicative of a distinct stock, each of which should be managed separately from a responsible fisheries and conservation viewpoints, unless it could be proven that all or some of the putative stocks were part of a patchy population (*sensu* Kritzer and Sale, 2004) with significant gene flow and a high likelihood of replenishment of extirpated sub-populations should they occur.

Sample Collection

As *P. kauderni* is not a protected fish species, and the collection areas do not yet have protected area status, no special collection or research permits were necessary. Adult Banggai cardinalfish *Pterapogon kauderni* (standard length SL $\geq$ 42 mm) were collected at random from six populations shown in Fig. 1 with a small fyke net called cang used by the local ornamental fishermen. The specimens were preserved in formalin (4% solution) for 12-24 hours then placed in 70% alcohol for transportation and storage. The site names, codes, geographical coordinates are listed in Table 1.

Table 1. Sample sites, number of specimens and their use

Sampling site (village)		Coordinates (WGS 84)		Number of Specimens		
Station Name	Codes	Latitude	Longitude	Total	GM	CM*
Popisi	PO - POA	S 1° 29' 57"	E 123° 30' 54"	33	33	30
Paisulimukon	PL - PLN	S 1° 33' 36"	E 123° 28' 42"	31	31	30
Tinakin Laut	TI - TIN	S 1° 36' 07"	E 123° 29' 24"	33	32	30
Monsongan	MO - MON	S 1° 37' 54"	E 123° 28' 53"	31	31	30
Tolokibit	TO - TOL	S 1° 42' 46"	E 123° 30' 58"	33	33	30
Matanga	MA - MAT	S 1° 42' 47"	E 123° 34' 58"	32	32	30
Total				193	192	180

GM = geometric morphometric study; CM = classical morphometric study
* Fin samples were also collected from these 30 samples for genetic analysis

Classical Morphometric Methods

The classical ichthyological morphometric parameters measured are shown in Fig. 2 along with the codes used and the description of each parameter.

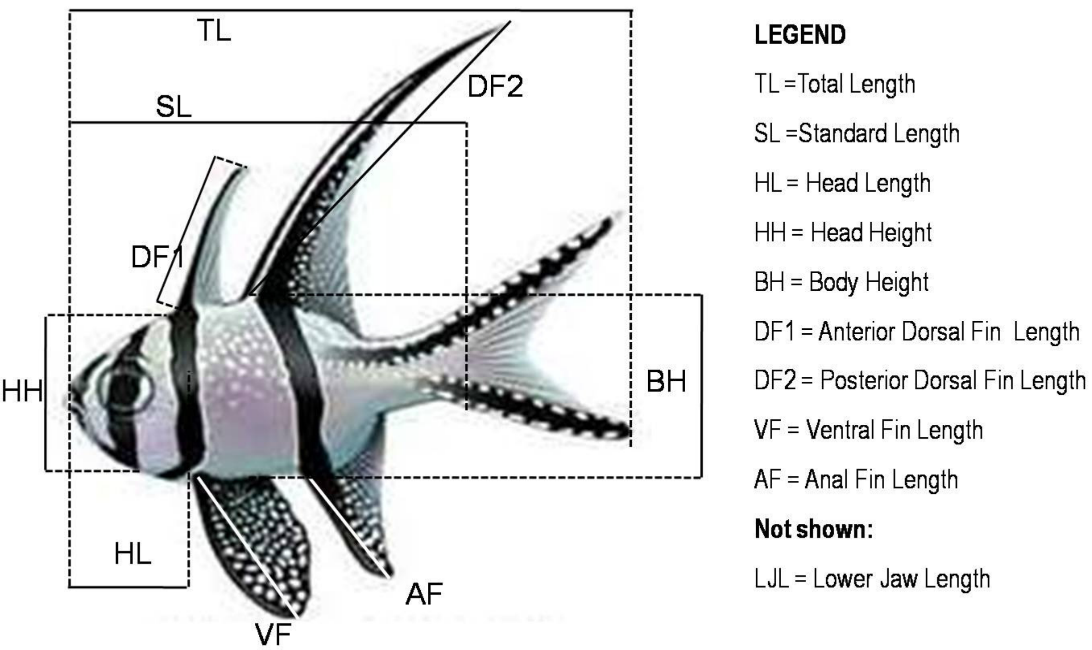


Figure 2. Classical morphological parameters measured

The parameters were measured using a digital calliper with a precision of ±0.01mm. Ratios of the eleven parameters measured were calculated, producing ten dimensionless parameters. Data on five of these parameters were available from a morphometric study on the *P. kauderni* Palu Bay introduced population (Ndobe *et al*., 2013), with the code TP. These data were compared with data from the six Banggai Island sites (Table 1). The dimensionless parameters analysed for the Banggai Island sites and those for which data from Palu Bay were used for comparison are listed in Table 2.

Table 2. Dimensionless morphometric parameters tested

Ratio	Banggai Island	Palu Bay (TP)	Ratio	Banggai Island	Palu Bay (TP)
TL/SL	3 sites	X	DF1/SL	X	X
HL/SL	X	X	DF2/SL	X	X
HH/SL	X	-	AF/SL	X	-
SL/BH (*aspect ratio*)	X	X	VF/SL	X	-
HH/BH	X	-	LJL/SL	X	-

The data were tabulated and statistical analyses implemented in Microsoft EXCEL 2007. The mean (average) and standard deviation were calculated for each parameter (ratio) by site (n = 30) and for the sample as a whole (N = 180). Results were analysed graphically.

Analysis of variance (ANOVA) was applied to each ratio for each pair of sites. F values were used to determine the level of significance for each pairwise comparison at 95% and (if appropriate) 99%. The results were analysed to produce matrices of variance between sites. An index of overall morphological variance (I_{mv}) between the populations of each pair of sites was calculated based on the number of ratios with significant or very significant variation between them using the equation:

$$I_{mv}\text{ (site i, site j)} = n_{\alpha=0.05\,(i,j)} + 2 \cdot n_{\alpha=0.01(i,j)}$$

where $n_{\alpha=0.05\,(i,j)}$ is the number of parameters (0-10) for which variance between sites i and j is significant at the 95% confidence limit ($F > F_{crit\,(\alpha=0.5)}$)

$n_{\alpha=0.01(i,j)}$ is the number of parameters (0-10) for which variance between sites i and j is significant at the 99% confidence limit ($F > F_{crit\,(\alpha=0.1)}$)

Imv was calculated for all 10 ratios, the 6 body shape ratios and the 4 fin length ratios. The results from these analyses were analysed descriptively, taking into account the various factors likely to be influencing the populations at each site. This analysis produced initial indications regarding population structure and stock boundaries as well as inferences drawn from the morphological characteristics of the introduced and endemic populations.

Geometric Morphometric Methods

Software and Digitising

The geometric morphometric analysis used the MorphoJ software (Klingenberg, 2011). Each specimen was photographed using a high resolution digital camera with standardised position (pinned to a polystyrene plaque) at a set distance from the camera lens with at least two repeats per specimen. Digitising and conversion to a file format compatible with MorphoJ was accomplished using two utilities: tps.dig and tps.util (Rohlf, 2012). Digitising was done at least twice for each photograph.

Landmarks

As for any geometric morphometric method, the first requirement was to establish a set of landmark points for the organism to be studied. As there were no previous studies on the Banggai cardinalfish *Pterapogon kauderni*, landmarks were chosen based on examples from other taxa and salient features of *P. kauderni* external morphology. The first set of landmarks comprised 17 points, greater than the ideal number of points (≤ 15, i.e. ≤50% of the lowest number of individuals per group). The final 14 landmark set used is shown in Fig. 3. Although contributing to visual representation of the variation in shape between populations by defining the caudal peduncle, trials showed that the elimination of three of the original 17 points (between 6 and 7 on lateral line and above and on the outline vertically above and below 14 in Fig.3) did not alter the statistical significance level of the two analyses used.

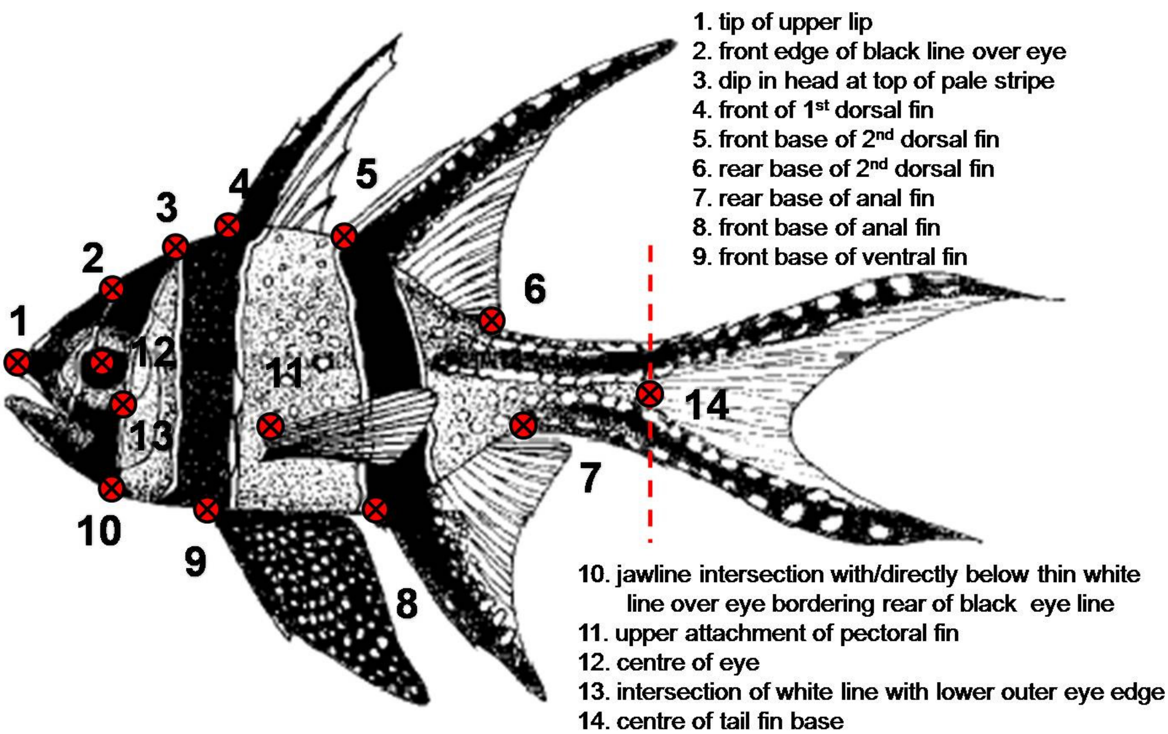


Figure 3. Landmark set for *Pterapogon kauderni*

Identifier Strings and Classifiers

In MorphoJ each digitised shape must have a unique identifier string. These strings were created during the digitation process in tps.dig and tps.util. Based on the characters at specific locations in this string, classifiers can be assigned in MorphoJ. The format given to each string and the classifiers defined and used in this study are given in Table 3.

Table 3. Identifier strings and classifiers

Item	Number and position (p) of characters	Value range or format	Definition/Remarks
Classifier string for each digitised image input	7 (p: 1 to 7)	XXXnnYm	XXX = 3 letter (A-Z) nn = 2 digit integer Y = L (left) or R (right) side m = repeat number (integer < 9)
Classifier "site"	3 (p: 1 to 3)	XXX	XXX = 3 letter (A-Z) site code (see Table 1)
Classifier "side"	1 (p: 6 or -2)	Y	Y = L (left) or R (right) side
Classifier "repeat"	1 (p: 7 or -1)	m	m = repeat number (integer from 1 to 3)
Classifier "fish" in the non-averaged (input) data set and Classifier string for data set averaged by "fish"	5 (p: 1 to 5)	XXXnn	XXX = 3 letter (A-Z) site code nn = 2 digit integer, assigned number of each fish (between 01 and 30 to 34 depending on the number of specimens sampled from each site)

Procrustes Configuration and Data Validation

Shape can be mathematically defined as the entire geometric information about a landmark configuration except its position, orientation, and scale (Dryden & Mardia 1998 *in* Klingenberg, 2011). Procrustes configuration in MorphoJ was used to eliminate the elements of position, orientation and scale through superimposition, rotation and scaling to unity, thus producing a series of centroids enabling comparison of shape alone. In MorphoJ the scale (size) element is retained in the data set (though not in the centroid itself) and can be used in certain analyses (not presented here) where size can be an important factor, for example as a proxy for ontogenetic differences. However in this study all individuals were within the adult size range and analyses concentrated on centroid shape.

The digitised data were examined for outliers. After 4 mis-numbered landmarks were corrected, one specimen (which had appeared abnormal from qualitative observation) remained an extreme outlier, and was not used in further analyses. Repeats for each specimen (photographs and digitising) were checked for consistency using the Procrustes ANOVA function, to ensure that measurement error was not significant compared to the variation between individuals. Once these steps were completed, the repeats for each individual were merged to produce a data set of centroids representing the 192 individuals from the 6 sites (*P. kauderni* populations) listed in Table 1.

Canonical Variate Analysis (CVA)

Canonical variate analysis (CVA) is a type of ordination analysis, which maximizes the separation of specified groups (Klingenberg, 2011). This analysis can be applied to several populations at once and was run for the whole data set with "site" as the classifier variable. The number of canonical variates is one less than the number of groups, and with six populations was therefore five. Outputs included statistical analyses with estimates of the significance (P values) and extent (Procrustes Distance) of between population (site) morphometric variation; graphic representations of the deformation function between the average shape of the whole sample (N = 192) and the average shape of each population; and plots of the spread of the specimens on axes representing any two of the canonical variates, essentially projecting two dimensions of the multi-dimensional shape space onto the X and Y axes. Between group (population) shape differences were considered significant if $P \leq 0.05$ and highly significant if $P < 0.0001$.

Discriminant Function Analysis (DFA)

The discriminant function analysis (CVA) with cross-validation indicates whether groups can be distinguished reliably (Klingenberg, 2011). This analysis can only be performed on two groups at one time and was run for each possible pairwise combination of sites. The discriminant function produced was validated using 1000 random permutations. Outputs included Procrustes distance, significance (parametric P value and P value for 1000 permutations), accuracy of the DFA in separating the original data (% separated) and in assigning each specimen correctly under 1000 random permutations (% correctly assigned). Pairwise shape differences between populations represented by the discriminant function were considered significant if $P \leq 0.05$ and highly significant if $P < 0.0001$.

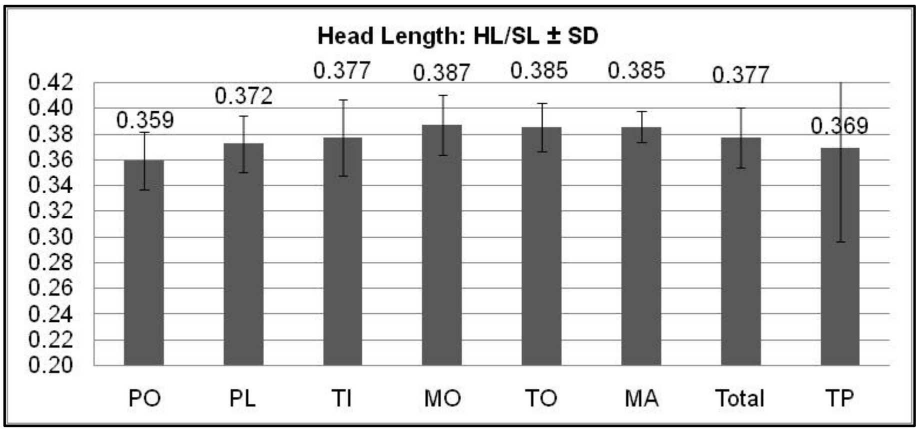
Synthesis

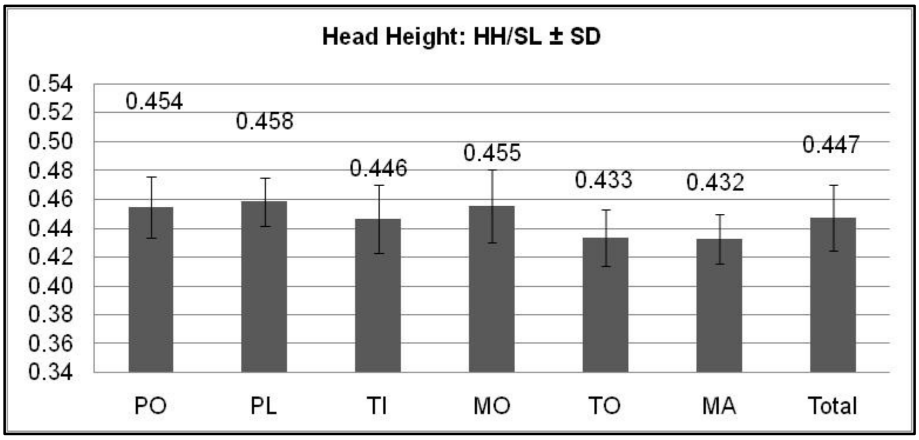
The results of the classical and geometric morphometric analyses were compared with each other and with the results of the genetic study described in Ndobe *et al.* (2012). The combined results were reviewed in the context of geophysical, ecological and socio-economic conditions including the ornamental fishery with respect to population structure, stock boundaries, management implications and future research needs.

RESULTS AND DISCUSSION

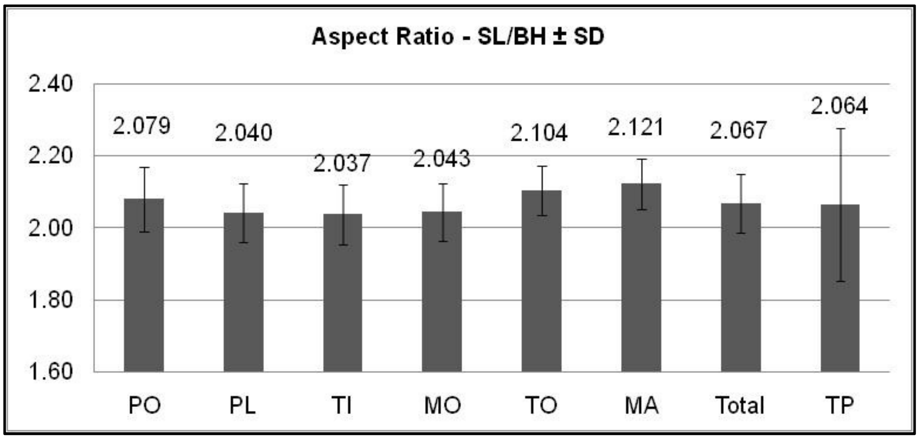
Classical Morphometric Results

The average values for each population of each of the 10 ratios are shown in bar graph form with standard error bars in Fig 4. The significance levels for each site pair for each of the 10 ratios are shown in Table 3, salient points are indicated in foot notes beneath the relevant graphs. The index of morphometric variance Imv values are shown in Table 4.

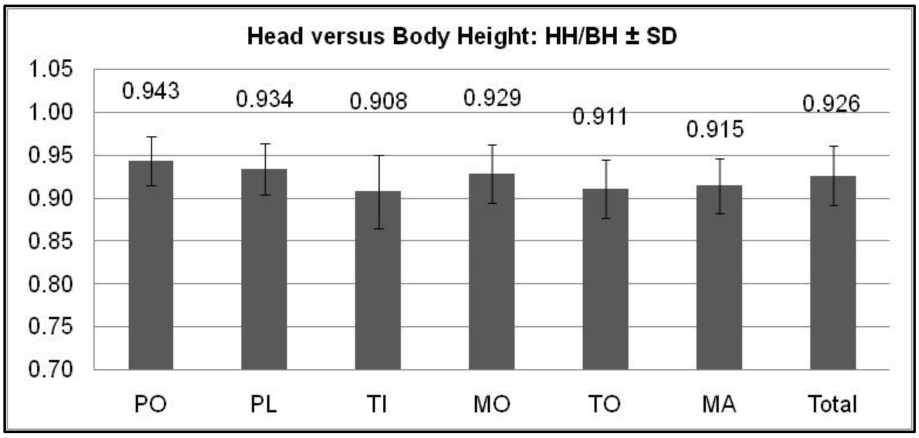




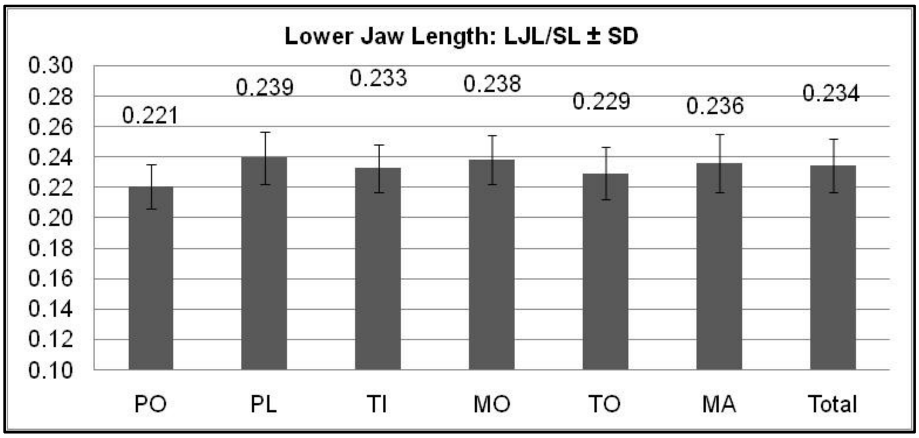
Popisi: shorter head length; Matanga and Tolokibit; lower (more pointed) head shape



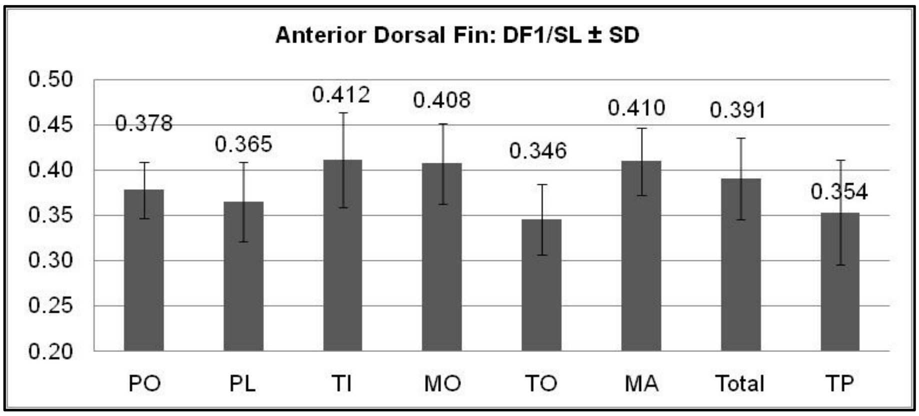
Indication of radiation of increasing aspect ratio from Tinakin Laut (TI)

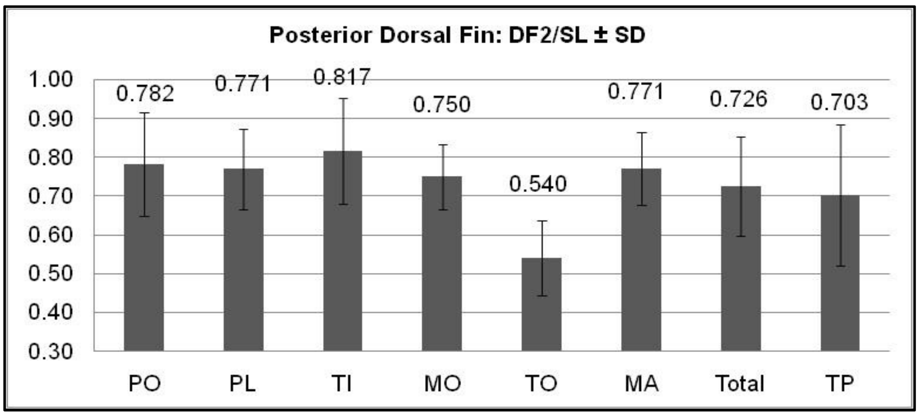


Heads more tapered in Tinakin, Tolokibit and Matanga



Shorter lower jaw length in Popisi consonant with shorter (and higher) head





Both dorsal fins shortest in Tolokibit and longest in Tinakin Laut

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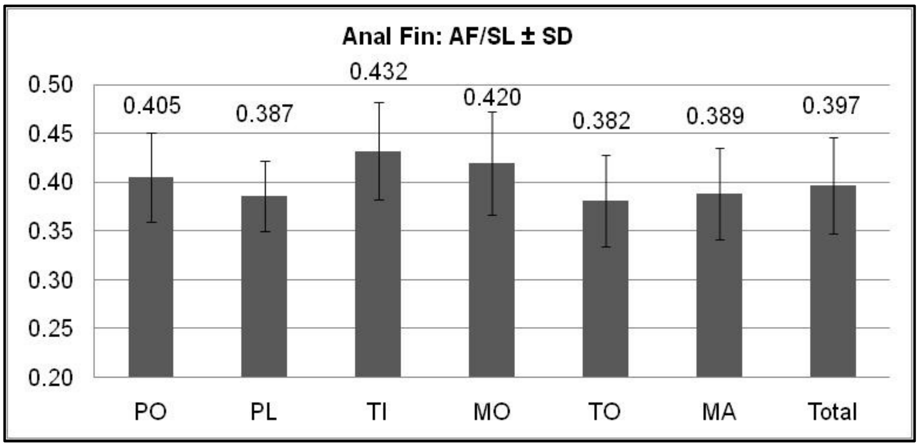
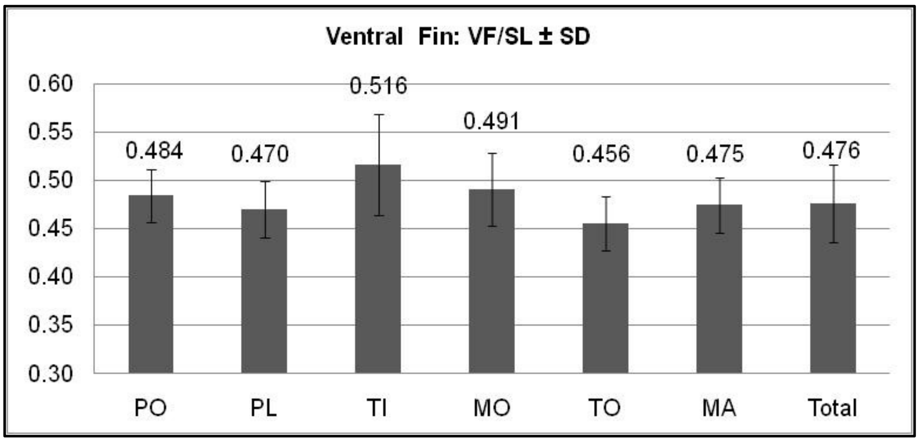
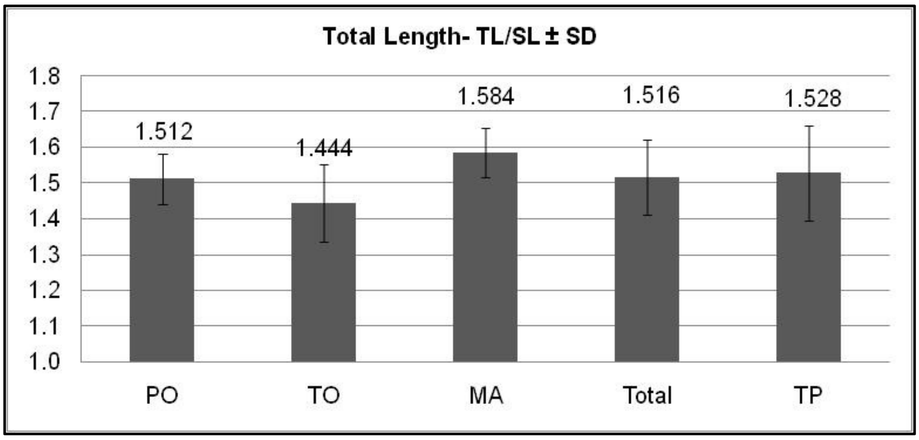




Similar variation in anal and ventral fins, shortest in Tolokibit, longest in Tinakin Laut



Tails longest in Matanga, shortest in Tolokibit, average in Popisi and Palu Bay

Figure 4. Ten morphometric ratios: average values per site ± SD

The data in Fig.4. show some specific characteristics for each population. Shorter fins in Tolokibit, longer fins in Tinakin Laut and Monsongan; a relatively elongated, streamlined shape in Matanga; short blocky heads in Popisi; and relatively large heads with relatively short fins in Paisulimukon. The Palu Bay population, founded through the release of fish from several populations including Tolokibit, was close to the total sample average for three head/body shape ratios, but had relatively short dorsal fins, possibly connected to this origin.

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Table 3. Pairwise ANOVA results for 10 Ratios and 6 sites

1. TL/SL	$F_{crit\ \alpha=0.05} = 4.043$; $F_{crit\ \alpha=0.01} = 7.194$; N = 75; n = 25				
	Paisulimukon	Tinakin	Monsongan	Tolokibit	Matanga
Popisi				*	**
Paisulimukon					
Tinakin					
Monsongan					
Tolokibit					**
	$F_{crit\ \alpha=0.05} = 4.007$; $F_{crit\ \alpha=0.01} = 7.093$; N = 180; n = 30				
2. HH/SL	Paisulimukon	Tinakin	Monsongan	Tolokibit	Matanga
Popisi	ns	ns	ns	**	**
Paisulimukon		*	ns	**	**
Tinakin			ns	*	*
Monsongan				**	**
Tolokibit					ns
3. BH/SL	Paisulimukon	Tinakin	Monsongan	Tolokibit	Matanga
Popisi	ns	ns	**	**	**
Paisulimukon		ns	ns	**	**
Tinakin			ns	**	**
Monsongan				**	**
Tolokibit					ns
4. HL/SL	Paisulimukon	Tinakin	Monsongan	Tolokibit	Matanga
Popisi	ns	ns	**	**	**
Paisulimukon		ns	*	*	**
Tinakin			ns	ns	ns
Monsongan				ns	ns
Tolokibit					ns
5. LJL/SL	Paisulimukon	Tinakin	Monsongan	Tolokibit	Matanga
Popisi	**	**	**	ns	*
Paisulimukon		ns	ns	*	ns
Tinakin			ns	ns	ns
Monsongan				*	ns
Tolokibit					ns
6.HH/BH	Paisulimukon	Tinakin	Monsongan	Tolokibit	Matanga
Popisi	*	**	*	**	**
Paisulimukon		*	ns	*	ns
Tinakin			ns	ns	ns
Monsongan				ns	ns
Tolokibit					ns

7. AF/SL	Paisulimukon	Tinakin	Monsongan	Tolokibit	Matanga
Popisi	ns	*	**	*	**
Paisulimukon		**	**	ns	**
Tinakin			ns	**	ns
Monsongan				**	ns
Tolokibit					**
8. VF/SL	Paisulimukon	Tinakin	Monsongan	Tolokibit	Matanga
Popisi	ns	**	**	**	*
Paisulimukon		**	*	ns	ns
Tinakin			*	**	**
Monsongan				**	*
Tolokibit					*
9. DF1/SL	Paisulimukon	Tinakin	Monsongan	Tolokibit	Matanga
Popisi	ns	**	*	ns	**
Paisulimukon		**	**	ns	**
Tinakin			ns	**	ns
Monsongan				**	*
Tolokibit					**
10. DF2/SL	Paisulimukon	Tinakin	Monsongan	Tolokibit	Matanga
Popisi	ns	ns	ns	**	ns
Paisulimukon		ns	ns	**	ns
Tinakin			ns	**	ns
Monsongan				**	ns
Tolokibit					**

346 N = total number of specimens in analysis; n = number of specimens per site

347 Table 4. Morphometric Variation Index I_{mv} for 6 sites

All 10 ratios	Paisulimukon	Tinakin	Monsongan	Tolokibit	Matanga
Popisi	3	9	12	14	16
Paisulimukon		8	6	10	10
Tinakin			1	11	5
Monsongan				13	6
Tolokibit					9
Body shape	Paisulimukon	Tinakin	Monsongan	Tolokibit	Matanga
Popisi	3	4	7	9	11
Paisulimukon		2	1	7	6
Tinakin			0	3	3
Monsongan				5	4
Tolokibit					2
Fin length	Paisulimukon	Tinakin	Monsongan	Tolokibit	Matanga
Popisi	0	5	5	5	5
Paisulimukon		6	5	2	4
Tinakin			1	8	1
Monsongan				8	2
Tolokibit					5

The classical morphometric data show very little difference between the Tinakin Laut and Monsongan populations with only one significant ratio, the relative length of the ventral fin (VF/SL). These two populations also have the highest within site variability for several parameters. Although the distance between these two sites in terms of coastline length is similar to that between several other pairs, and the shallow water environment is somewhat different, there is no obvious break in *P. kauderni* habitat between these two sites with reef flats of varying width extending along the intervening coast. Despite the extreme philopatry exhibited by *P. kauderni* based on behavioural and genetic studies (Kolm *et al.*, 2005) and inferred from genetic population data (Vagelli *et al.*, 2009), it is likely that some natural migration and therefore genetic exchange does take place.

Furthermore, intermixing between these two sites could have been facilitated by the prevalent fishing and trading patterns in the late 1990's to around 2004. In Bone Baru where the ornamental fishermen have tended to exploit a large number of fishing grounds, so that fish from many sites have been released in substantial numbers. However when the trade was active in these two villages, the Monsongan and Tinakin Laut ornamental fishers tended to catch most fish in or close to their own village however it is possible that there have been unsold fish from other sites released, especially from Tinakin Laut and possibly Tolokibit at Monsongan and from Monsongan and possibly Paisulimukon at Tinakin Laut.

The greatest difference is between the populations at the two extremities of the Banggai cardinalfish distribution, Popisi in the north and Matanga in the southeast. This could be related to distance, a hypothesis for which the matrices in Table 4 show some support, with either a latitudinal gradient or radiation from the Tinakin Laut/Monsongan area. Alternatively, habitat could be a factor. Popisi is the most sheltered of the six sites, while Matanga is the most exposed. The relatively hydrodynamic body and long fins of the Matanga population could be an adaptation by natural selection on genetic traits or influence on individual growth. Conversely the relatively large chunky head shape exhibited by the Popisi population would not be a disadvantage in the calm waters of the bay and might provide other advantages such as greater capacity for mouthbrooding.

Several ratios are significant to a level between 90% and 95%, in particular between Popisi and Paisulimukon. While not considered statistically significant, biological and ecologically these differences could be of significance and indicate that the two populations are more distinct morphometrically than might appear from Table 3 and Table 4.

Overall, the classical morphometric data indicate five possible stocks: Popisi, Paisulimukon, Tinakin/Monsongan, Tolokibit and Matanga. The comparison with the Palu

Bay population reinforces the possibility of mixed origins in the founder population. Despite the statistical significance of variation in some of the morphometric ratios at a population level, the level of overlap between individuals from different sites means that none of these ratios can be used as a marker to identify the origin of a particular individual, such as the lower jaw length and dorsal fin which enabled Uglem *et al*. (2011) to distinguish (with a confidence level in excess of 95%) between wild cod (*Gadus morhua*) and escapees from aquaculture facilities.

Geometric Morphometrics

Canonical Variate Analysis (CVA)

The eigenvalues and variance explained by each of the five canonical variates are shown in Table 5. These values indicate that the first canonical variate (CV1) explains over half of the total variance and the first three variates explain almost 90% of total variance.

Table 5. Canonical variate eigenvalues and proportion of variance explained

Canonical Variate (CV) Number	Eigenvalue	% of variance explained	Cumulative % of variance explained
1	3.11095	57.088	57.088
2	1.0215	18.745	75.833
3	0.76136	13.972	89.805
4	0.30743	5.642	95.446
5	0.24816	4.554	100

Graphic representations of the first three canonical variates are shown in Fig. 5. The points show the average position (for all 192 specimens) of each landmark, and the lines represent the direction and relative magnitude of the deformation from this average shape represented by the canonical variate. The canonical variates CV1 and CV3 both seem to relate quite strongly to aspect ratio while CV2 seems more related to head shape.

Plots of the 192 individuals with 90% confidence ellipses of the six sites (populations) for two dimensional plots with X or Y axes of CV1, CV2 and CV3 are shown in Fig 6. CV5 and CV5 (not shown) did not show any significant separation between sites and seem to relate to individual rather than population or site-based characters.

The value of $P < 0.0001$ for the between site analysis of variance (ANOVA) for the total sample ($N = 192$) shows that there is significant variance between populations based on the canonical variates. The pairwise between site P values are shown in Table 6, and show significant Procrustes distance between all site pairs except Tinakin Laut and Monsongan (TIN-MON).

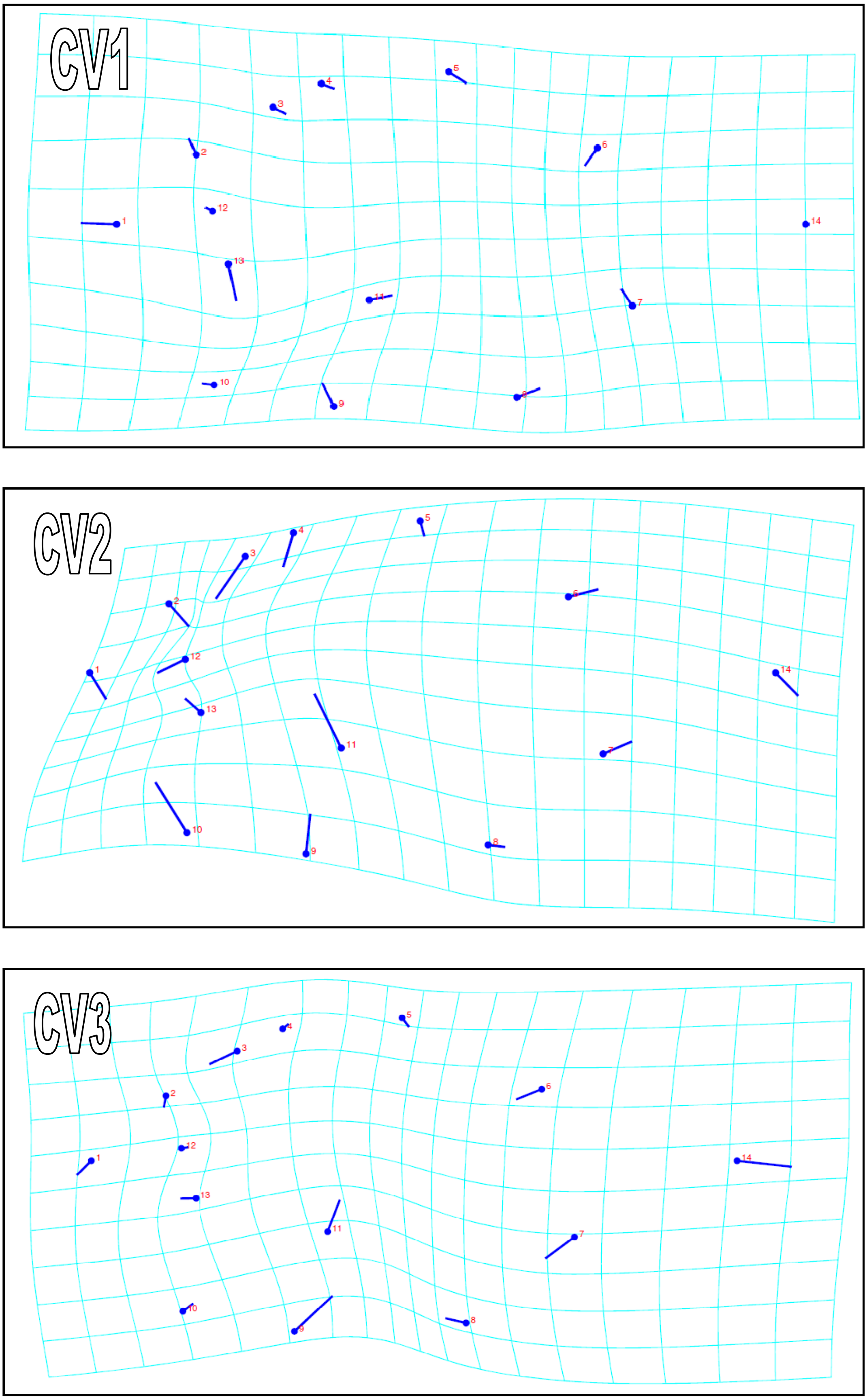


Figure 5. Deformation grid and direction of deformation from average shape represented by the first three canonical variates (CV1, CV2, CV3)

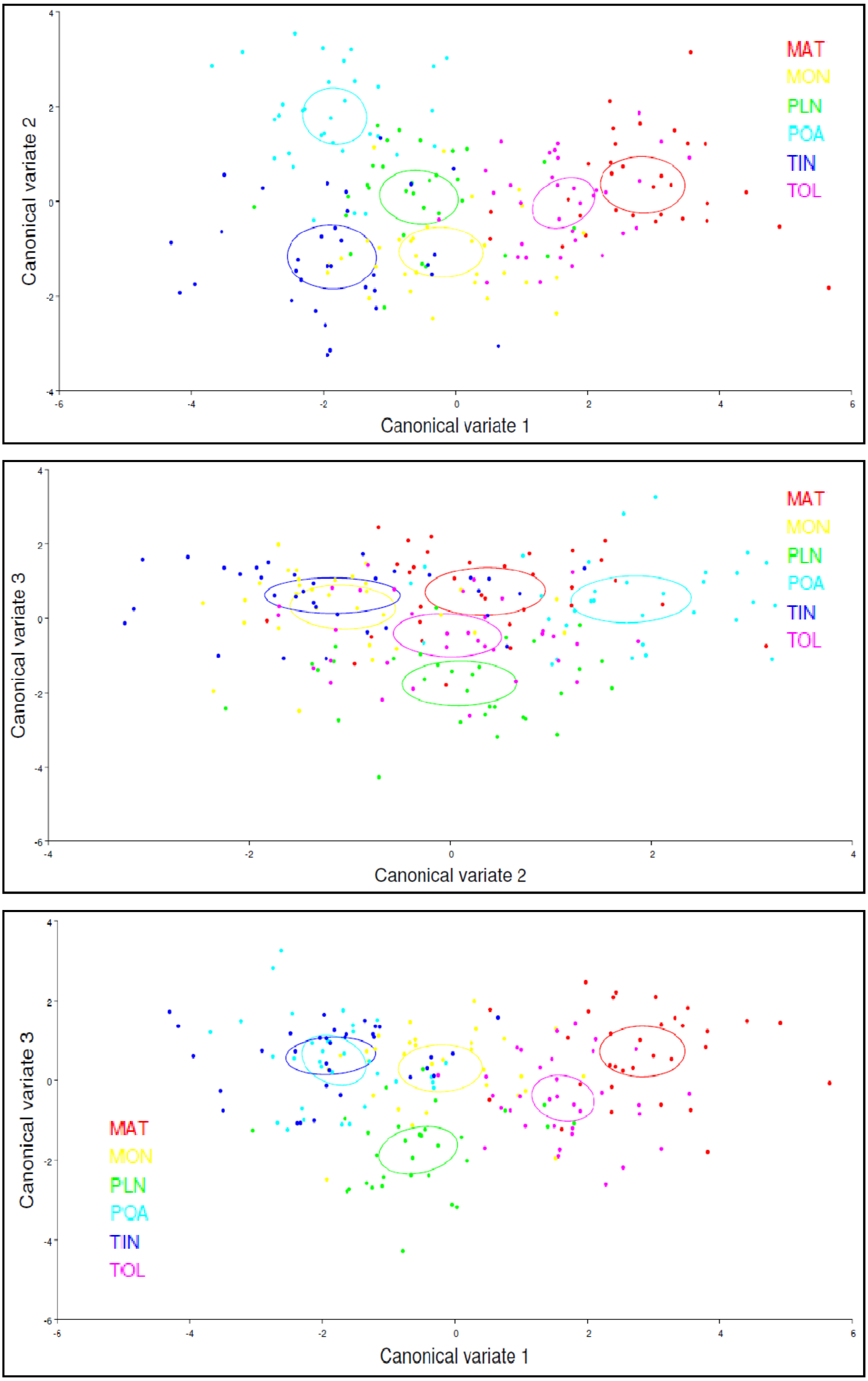


Figure 6. Distribution of the six study sites on the three first canonical variate axes: CV1/CV2 (top), CV1/CV3 (centre) and CV2/CV3 (bottom)

Table 6. Significance of between site Procrustes Distance

Pairwise P values (below diagonal) and significance level (above diagonal)						
	POA	PLN	TIN	MON	TOL	MAT
POA		**	**	**	**	**
PLN	<0.0001		*	ns#	*	**
TIN	<0.0001	0.0043		ns	*	**
MON	<0.0001	0.0619	0.2067		*	*
TOL	<0.0001	0.0007	0.0015	0.0324		*
MAT	<0.0001	<0.0001	<0.0001	0.0023	0.0228	

* significant; ** highly significant; ns non statistically significant; # $P \leq 0.07$, in the range sometimes considered significant in biological or ecological terms (Klingenberg, 2012)

The plots in Fig. 6 show that Matanga and to a lesser extent Tolokibit each exhibit considerable difference from the other 4 sites and from each other with respect to CV1. With respect to CV2, there are three groups: Popisi is markedly different from the Monsongan-Tinakin sites which almost wholly overlap while the other three sites (Paisulimukon, Tolokibit and Matanga) show substantial overlap. The CV3 axes show Paisulimukon as being well separated from the other 5 sites. Together the plots show that the only two sites not markedly separated from all other sites on any of the three axes are Tinakin Laut and Monsongan.

Discriminant Function Analysis (DFA)

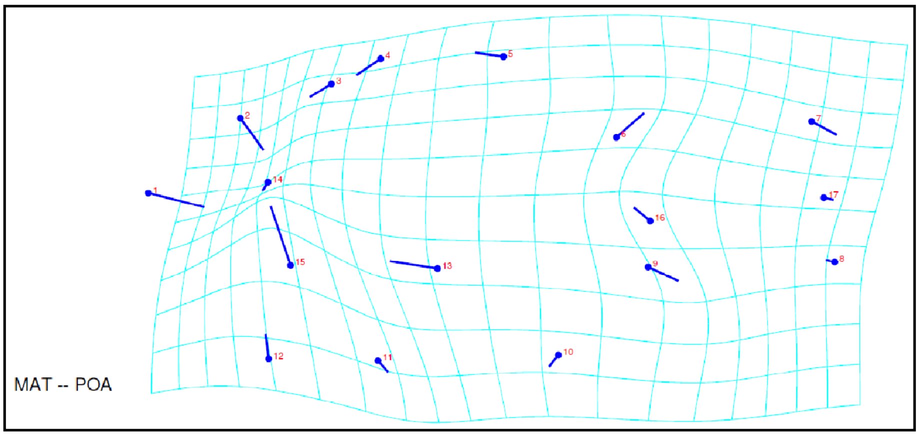
Unlike the canonical variate analysis (CVA), the discriminant function analysis (DFA) in MorphoJ can only be run for two groups, in this case for the 15 possible site pairs. The results of the parametric analysis which sets up the discriminant function (DF) and of the validation assignment tests of each DF (with 1000 random permutations) are shown in Table 7. Examples of the deformation or transformation represented by the DF are shown in Fig. 7. Examples of the distribution of the individuals within each population relative to the discriminant function are shown in Fig. 8. Examples of the results of the DF validation assignment tests are shown in Fig. 9.

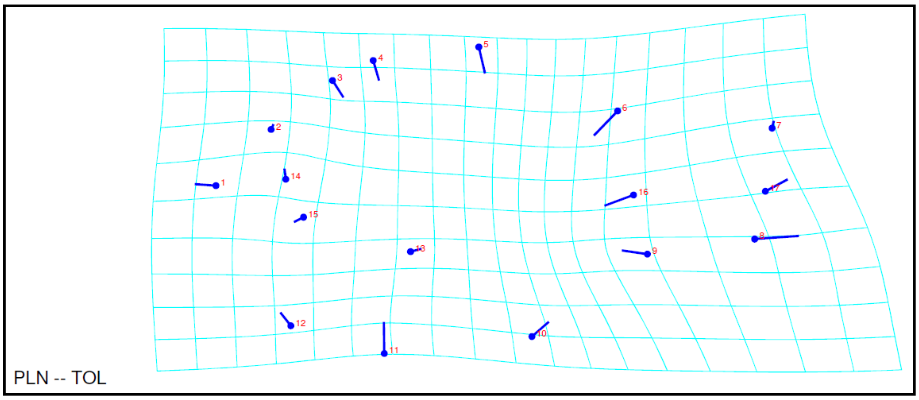
The data in Table 7 show that all site (population pairs are significantly different under the parametric discriminant function produced, with 95% to 100% of fish being described as belonging to the correct site. The level (%) of successful attribution under the validation test varied, but was statistically significant for 5 site pairs and highly significant for 9 site pairs. The highest validation score (98%) was for Popisi-Matanga, while Monsongan-Tolokibit had the lowest significant validation score (72%). However one site pair, Tinakin Laut-Monsongan, was not significantly discriminated under the validation test.

Table 7. Pairwise DFA analysis significance, discrimination and validation

Site (Population)	P value and significance: Parametric (DF)	P value and significance: Validation (1000 random permutations)	Discriminant power of the DF (%)	Correct validation assignment (%)
POA-MAT	<0.0001 **	<0.0001 **	100	98
POA-TOL	<0.0001 **	<0.0001 **	100	94
POA-MON	<0.0001 **	<0.0001 **	100	87
POA-TIN	<0.0001 **	<0.0001 **	95	76
POA-PLN	<0.0001 **	<0.0001 **	98	78
PLN-MAT	<0.0001 **	<0.0001 **	100	87
PLN-TOL	<0.0001 **	<0.0001 **	95	80
PLN-MON	<0.0001 **	0.0010*	100	89
PLN-TIN	<0.0001 **	<0.0001 **	97	86
TIN-MAT	<0.0001 **	<0.0001 **	100	95
TIN-TOL	<0.0001 **	0.0010*	100	95
TIN-MON	<0.0001 **	0.0840 ns	97	87
MON-MAT	<0.0001 **	0.0020*	100	92
MON-TOL	<0.0001 **	0.0380*	97	72
TOL-MAT	<0.0001 **	0.0230*	100	76

** = highly significant; * significant; ns not statistically significant





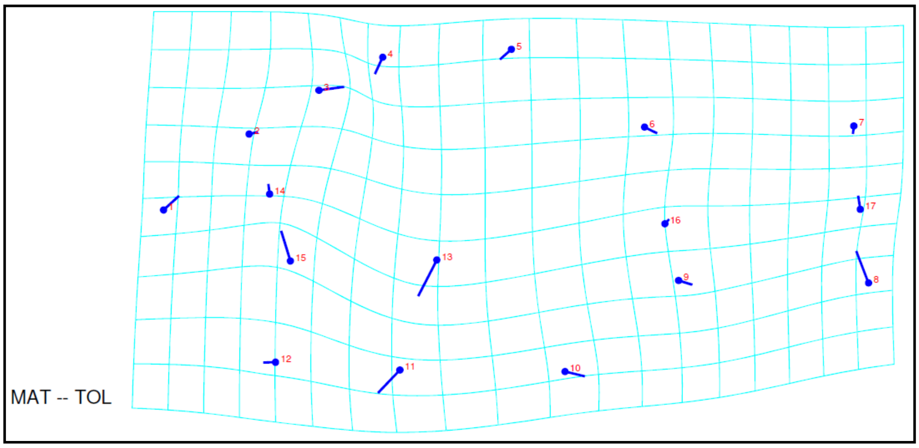


Figure 7. Graphical representation of the distortion represented by the Discriminant Function (DF) for 3 study site pairs: MAT-POA; PLN-TOL; MAT-TOL (with x 6 magnification)

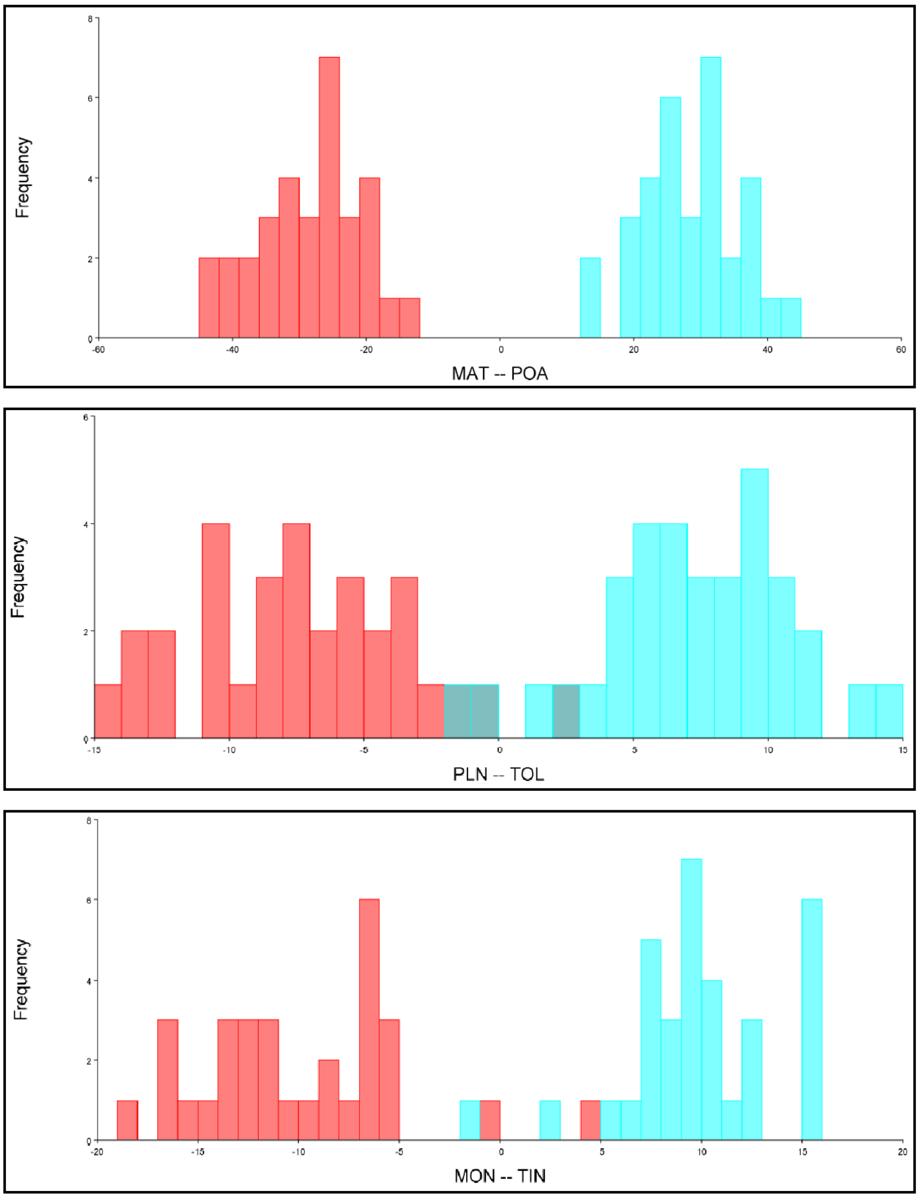


Figure 8. Descriminant Function (DF) score distribution 3 site pairs:
MAT-POA (top); PLN-TOL (centre) ; MON-TIN (bottom)

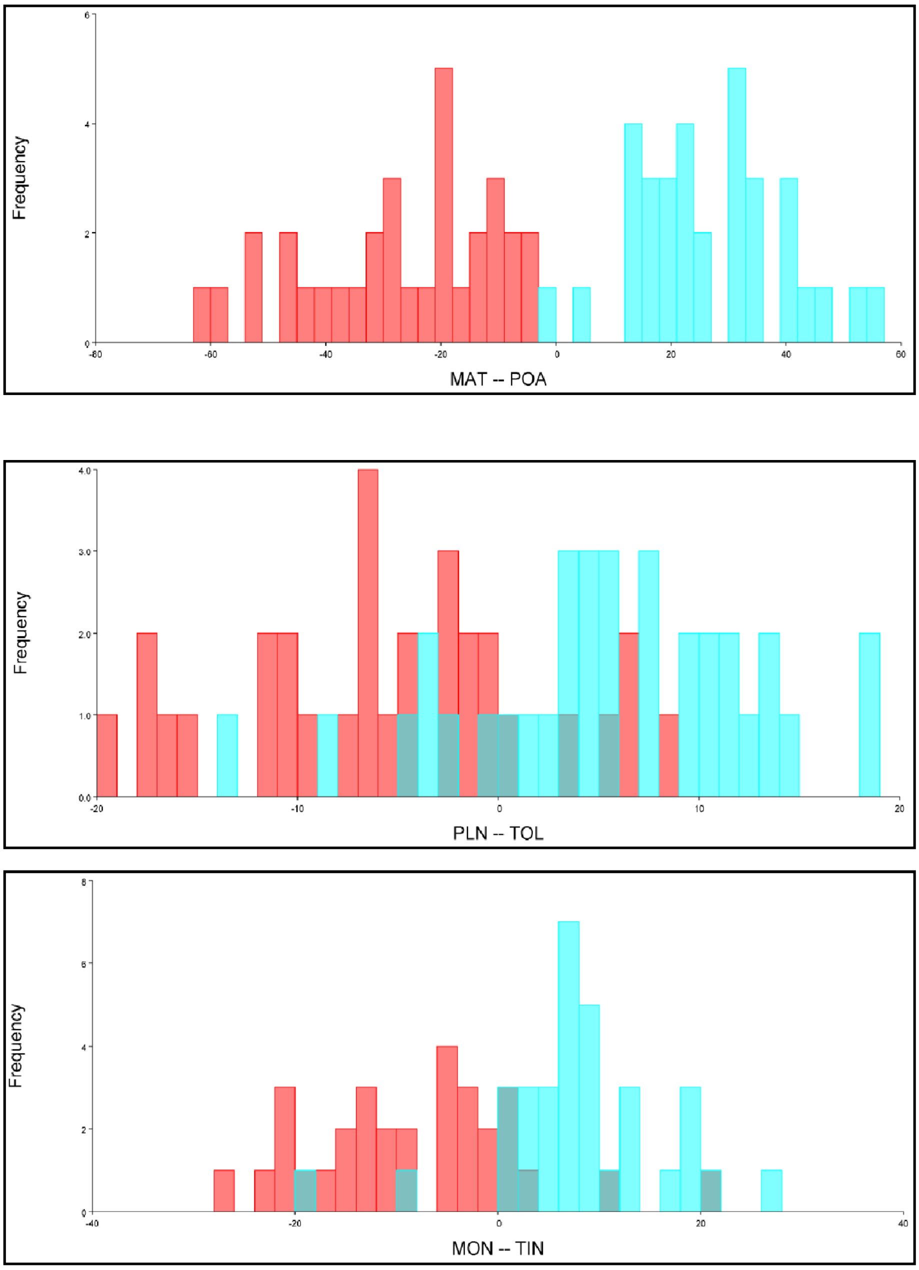


Figure 9. Discriminant Function (DF) cross-validation scores for 3 site pairs: MAT-POA (top); PLN-TOL (centre); MON-TIN (bottom)

Synthesis – morphometric and other data

The two morphometric methods used provide different and complimentary information on population characteristics, but both produce the same result in terms of stock identification, pointing to 5 stocks, four of which are likely to be closed or very nearly closed populations (Matanga, Tolokibit, Paisulimukon and Popisi) and one of which would seem to display the characteristics of a metapopulation (Tinakin Laut-Monsongan). This result fits in well with geophysical data on potential breaks in habitat (Ndobe *et al*., 2012), and genetic data from Hoffman *et al*. (2005) and Vagelli *et al*. (2009) and reinforces the conclusion drawn from

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genetic analysis on the same sites and sample specimens (S. Ndobe, unpublished). A map of the proposed *P. kauderni* stock boundaries around Banggai Island is shown in Fig. 10.

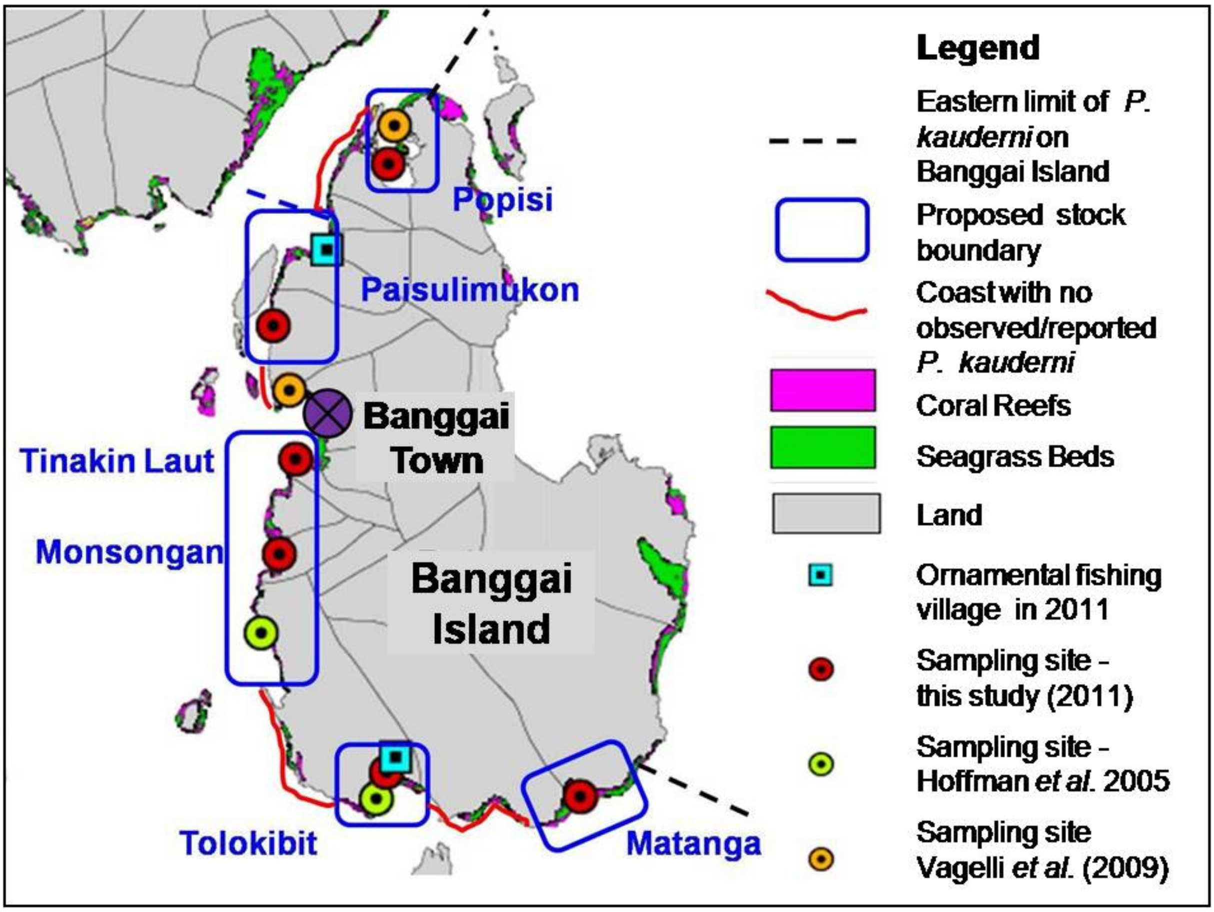

Figure 10. Five proposed *P. kauderni* stocks around Banggai Island

Many factors or processes could contribute to or cause the observed differences between populations and the evolution of the proposed stocks. One possibility could be radiation for example if a founder population had been established and then slowly spread (N-S or S-N or N and S from an intermediary point) at a period when sea levels were lower and current habit was connected by shallow coastal habitat corridors. The populations might then have become separated and evolved differently when sea levels rose. Such an explanation for population structure has been proposed for other fishes, for example the striped snakehead *Channa striata* (Jamaluddin *et al.*, 2011).

It is possible and even likely that trade has resulted in some anthropogenic movement of individuals from one site (and possibly population or stock) to another, mainly from Banggai or Bandang Islands as previously mentioned. Between site environmental differences may have driven site-specific selection and evolution, for example exposure to waves and currents, habitat typology and microhabitat availability. For example the relatively elongated form and long tails/fins in Matanga may be an adaptation to the exposed nature of the site. In the east monsoon powerful waves and strong currents tend to reduce the *P. kauderni* population in most years, so that a better swimming ability would be an advantageous trait likely to increase survival and reproductive success, despite possible trade-off such as reduced ability to store energy for mouthbrooding or potentially reduced volume of the buccal cavity and hence brooding capacity of males.

Another theoretical possibility is that of remnant populations or stocks. The Banggai Archipelago was originally a fragment from the Australasian tectonic plate which moved north. It is possible, even probable that *P. kauderni* evolved and spread across this plate, much of which would have been shallow seas suitable as habitat. Rising sea levels could have reduced this widespread population with at least some genetic connectivity to a number of relatively small and increasingly isolated sub-populations or stocks well before historical times. Extirpations and subsequent recolonising radiations could have occurred.

Further research might be able to elucidate the biogeography and genetic population structure as well as morphometric characteristics of *P. kauderni*. For example, multidisciplinary evolutionary studies of the area; genetic research on the phylogeny and ancestral origins of *P. kauderni*; genetic populations studies using more than two microsatellites or other genetic methods (e.g. sequencing); studies of other morphometric and meristic characters including colour and patterns, which appear to vary between at least some sites; in-depth long-term studies on reproductive success, recruitment and individual movement patterns; and combined morphometric and genetic studies in other areas of the P. kauderni distribution.

Based on the precautionary approach to fisheries resource management now widely advocated, the data already available provide a basis for managing the ornamental fishery in the waters around Banggai Island and to inform the zonation of Banggai Island for *P. kauderni* conservation in the context of the District MPA. Indeed zonation options based on this and other research have been produced (Ndobe, unpublished) using the MARXAN MPA planning software (Ball and Possingham, 2000).

Despite already having an (untenanted) office in Bone Baru since 2009, the Banggai Kepulauan District MPA was still in the planning stage in early 2013 when the District was further subdivided. The new Banggai Laut District with the town of Banggai on Banggai Island as its capital, comprises the majority of the *P. kauderni* endemic distribution in the southern part of the archipelago. Clearly the District MPA will have to be reviewed. Options include establishing two District MPAs, a cross-boundary MPA under higher level jurisdiction (provincial or national), or abandonment. Either of the first two options would provide an opportunity to improve the effectiveness of the original design, which was poor from several aspects and in particular from a *P. kauderni* conservation point of view (Ndobe *et al.*, 2012).

CONCLUSION

Both morphometric methods used show significant differences between the six *P. kauderni* populations studied. The population structure indicated conforms with genetic study results, and strongly indicate the presence of 5 stocks in the waters around Banggai Island. We propose that these stocks should be treated as management units and thus as a basis for *P. kauderni* management in the context of the ornamental fishery and in conservation management, in particular the process of reviewing and implementing the District MPA.

We recommend further research to improve understanding of the phenomena causing the observed differences, as well as similar studies (ideally combining genetic and morphometric analyses) in other sites across the *P. kauderni* distribution. We consider that application of morphometric geometrics would be beneficial in other aspects of *P. kauderni* bioecology, for example seeking means of differentiating female and (non-brooding) male Banggai cardinalfish as well as applications to other species in Indonesia, where the method is still little known.

ACKNOWLEDGMENTS

The authors wish to thank all who in any way supported the research and the writing of this article. We are aware that this first version, and could be significantly improved. We welcome and express our gratitude in advance to all who may offer constructive criticism and advice on this pre-print article, which we hope to publish with PeerJ as a peer reviewed research article.

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