Measuring the historical conservation status of dragonet fishes in Tosa Bay, Southwestern Japan: ecological and genetic approach

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ABSTRACT

Dragonets are one of the dominant species in Tosa Bay, Southwestern Japan. However, until now, there is no baseline information on the conservation status of its species and populations. This study gathered genetic and ecological data and information to analyze and measure the historical conservation status of dragonets in the Bay. Quantitative values were converted into qualitative ranges to measures the conservation status of dragonet species. Eight dragonet species/populations were found to be in stable condition in the early 1990s, namely: *Callionymus planus* Ochiai, 1955; *Callionymus lunatus* Temminck and Schlegel, 1845; *Callionymus curvicornis* Valenciennes, 1837; *Callionymus japonicus* Houttuyn, 1782; Callionymus *enneactis* Bleeker, 1879; *Synchiropus altivelis* (Temminck and Schlegel, 1845); *Repomucenus virgis* (Jordan and Fowler, 1903); and *Repomucenus huguenini* Bleeker, 1858. Others were globally endangered and rare (*Callionymus draconis* Nakabo, 1977), locally highly vulnerable (*Callionymus valenciennei* Temminck and Schlegel, 1845; *Callionymus beniteguri* (Jordan and Snyder, 1900), locally vulnerable (*Callionymus formosanus* Fricke, 1981; *Bathycallionymus kaianus* (Günther, 1981)), and globally highly vulnerable (*Callionymus sokonumeri* Kamohara, 1936). The information gained in this study provided baseline knowledge on the historical species risk status of dragonets in Tosa Bay, which can be used as a basis for future studies. It also provided some scientifically-based options for managing biodiversity in a defined spatial management unit, which is applicable to e.g., marine protected areas, parks, bays, islands, lakes, etc.

Keywords: biodiversity indicators, risk assessment, spatial management unit

The Palawan Scientist, 13(2): 37-52

INTRODUCTION

Every species on earth has its special value as conferred by its evolutionary history, unique ecological roles (Primack 1995), and beauty. Loss of genetic diversity reduces future evolutionary options, and high genetic diversity variation within populations is positively related to fitness (Meffe and Carroll 1994). The human beings are dependent on other species to exist, as species depend on other species; thus, what is bad for biological diversity is bad for humans. However, in-spite of their value, vast numbers of species have declined rapidly, to some point of untimely extinction at a rate that far exceeds the rate of species replacement (Primack 1995). Cheung et al. (2005) stress that effective conservation of threatened species requires timely identification of vulnerable species, and careful measurement of environmental trends and progress will provide a foundation for effective policymaking (Garcia 1996). As such, there is a need to identify which species are at risk of extinction (Dulvy et al. 2004) and which populations are threatened to form a basis to decide suitable management options. Furthermore, the United Nations Environmental Programme (UNEP) Key Marine and Coastal Biodiversity uses data sets to monitor biodiversity changes through time or identify areas of high biodiversity value (Martin et al. 2014). Shin et al. (2005) agree that several indicators may be needed to track the state of several components and attributes of biodiversity.

To this effect, the Food and Agriculture Organization of the United Nations has established the international framework for the protection and sustainable development of the marine and coastal environment and its resources by laying out the rights and obligations of states (FAO 1992). This provision obliges the coastal states to undertake measures to maintain biodiversity, including a marine biodiversity survey and an inventory of endangered species and critical marine habitats. Consequently, Yankova et al. (2014) and Pešić et al. (2021) prepared a list of rare and endangered fish species of the Adriatic Sea and the checklist of the marine fishes in the Black Sea, as well as their current conservation status, respectively.

Hence, it is necessary to develop biodiversity indicators defined as variables, pointers, or indices of a phenomenon (Garcia et al. 2000) which are widely used for environmental reporting, research, and management support (Spellerberg 2005). Twenty-five percent of the ecologists choose indicators that assess a combination of local abundance, ecological significance, or conservation status (Siddig et al. 2016). For better accuracy, indicators compose of different disciplines are needed to cover more population components and traits within a defined management area. Garcia (1996) stresses that the scope of indicators should encompass both the sustainable resources, commodities, services and important societal factors derived from the system. In addition, Jennings (2005) considered an ecosystem (or more realistically a spatial management unit) with components (e.g. population or species) and attributes (e.g. diversity, abundance, trophic structure). On the other hand, the Indicators for Sustainable Development of Fisheries (ISDF) emphasizes species and genetic diversity loss as biodiversity criteria (Garcia 1996). Meanwhile, the Traffic Light (TL) approach integrates multiple quantitative and qualitative criteria to assign management responses to fisheries management (Caddy 1999, 2015).

While genetic approaches became popular with the conservation of rare species (e.g. Ashbaugh et al. 1994; Primack 1995), Gaston and Lawton (1990) and Huston (1990) use correlations between population ecology and rare species to determine conservation priorities. Burgman et al. (1993) use such qualitative categories as abundance, spatial distribution, geographic range, frequency of occurrence indicators to assess species or population status, while Kirpichnikov (1992) correlated the genetic variability to population size. It is thus important to work on the elements of biodiversity in a locality, involving the combination of species diversity, genetic diversity, ecological traits, microhabitat, and phylogeny.

Callionymids are one of the dominant species in Tosa Bay, Japan. Despite the numerous biodiversity studies conducted on different species in the area, there is still no information on the conservation status of the species. Therefore, this study aims to determine the historical conservation status of identified populations and species of dragonets in Tosa Bay and Uranouchi Inlet, Japan, using the combination of two parameters (ecological and genetics) and infer on the historical conservation status of the species (rare, stable, or vulnerable). This information could serve as a historical baseline on the conservation status of various species of dragonets in the Bay.

METHODS

The information on the depth distribution of 12 dragonets species collected in Tosa Bay and Uranouchi Inlet, Japan, was from the work of the authors (Gonzales and Taniguchi (1997b), while genetic information of dragonets in Tosa Bay was based on the allozyme study of Gonzales et al. (1997a). The correlation coefficient (2-tailed test: Spearman's rho) of genetic and ecological indicators was used to determine the relationships among the indicators of the conservation status of dragonet species and populations.

Measurement of Historical Conservation Status using the Population Ecology and Genetic Variation

The varying degrees of the conservation status of different populations and species of dragonets in Tosa Bay and Uranouchi Inlet were measured based on the quantitative categories, both from their population ecology and genetic variations where quantitative values were converted into qualitative ranges. Population ecology (four qualitative categories) and genetic variation (two qualitative categories) were used as indicators to assess the risks of population and species (Table 1). An arbitrary score (points) ranging from zero to two were designated for each of the qualitative category ranges to estimate the degree of risk of each population (Table 1). The minimum score for each species in all categories were six points (Table 2) and a maximum of 12 to be considered stable.

Table 1. Arbitrary scores used in different indicators in determining the conservation risk of dragonets population and species in Tosa Bay and Uranouchi Inlet, Japan. Polymorphism (P) value average and lower margin were based on Kirpichnikov (1992). *other populations of the species can be found in the far seas as in the waters of Indonesia and Australia, and the Indian ocean; **found in Japan or waters adjacent to it; e.g. the South China Sea, East China Sea, and in waters off the Korean Peninsula; +reported only in Tosa Bay or the Pacific Coast of Southern Japan.

Arbitrary Score	Population Biology				Genetic Variation	
	Abundance (ind mo^{-1})	Depth Distribution (no. of depth zone)	Geographical Range	Frequency (frequency of occurrence in the trawl catches)	Polymorphism (P)	Heterozygosity (H)
2	abundant (> 30)	widespread (> 5)	widespread*	ubiquitous (> 60%)	high (P > 20%)	high (H> 5%)
1	average (15-30)	average (3-5)	domestic**	average (40-60%)	average (P between 8-20%)	average (H between 2-5%)
0	rare (< 15)	restricted (< 3)	endemic+	scarce (< 40%)	low (P < 8%)	low (P < 2%)

The species *Callionymus Draconis* Nakabo, 1977*, Callionymus valenciennei,* and *Foetorepus masudai* Nakabo, 1987 were not included in the point system due to a lack of data in some categories. This study is limited to dragonet species found only in Tosa Bay and the adjacent Uranouchi Inlet (southwestern Japan – as a spatial management unit). Other dragonet species found in other areas of Japan, like the *Callionymus ornatipinnis* Regan, 1905 in northern Japan (Awata et al. 2010), were not included.

Table 2. The score points used to assess the conservation status of dragonets population and species in Tosa Bay and Uranouchi Inlet, Japan. *Based from Burgman et al. (1993).

Score points	Conservation status	Interpretation
≥ 50% (6)	Stable	Population not at risk
< 50% (5)	At risk	Unstable, may become vulnerable if no management intervention is in place
25–42% (3-5)	Vulnerable	*Population prone to be endangered about 75–100 years if factors tending to push the population into decline continue to operate.
≤ 25% (3)	Highly vulnerable	*Population prone to be endangered at about 50-75 years if factors tending to push the population into decline continue to operate.

Determination of Genetic Variation and Species Identification

The genetic heterozygosity and polymorphism used in this study was from Gonzales et al. (1997a). Fish samples were collected in Tosa Bay, Uranouchi Inlet, and nearby fish market (Figure 1). Fish sample tissues used for the electrophoretic analysis were skeletal muscle, liver, and eye. Horizontal starch-gel electrophoresis and staining procedures followed Sumantadinata and Taniguchi (1982), with slight modifications. The buffer systems used were citric acid-aminopropyl morpholine (C-APM) at pH 6.0 and Tris-citrate (T-C) at pH 8.0.

The gene nomenclature for protein-coding followed Shaklee et al. (1990). Allelic frequencies were calculated following Allendorf and Ferguson (1990). Nei (1972) genetic distance ($D = \log_e I$) was calculated for each species pair, using all loci at which genotypes were scored for both species. Test of conformance to expected Hardy-Weinberg proportions was carried out by conventional chi-square tests with Yate's correction (Pasteur et al. 1988).

All scientific names of dragonets followed the FishBase (Froese and Pauly 2021) and were validated using the work of Fricke et al. (2020) and WoRMS database (WoRMS Editorial Board 2021).

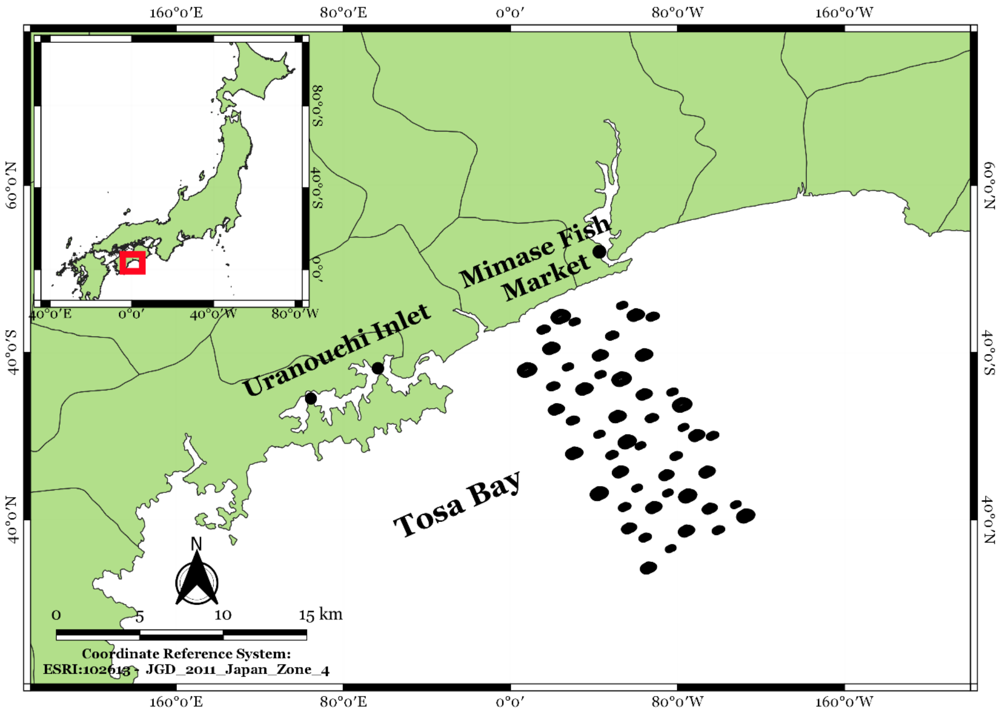

Figure 1. Map of Tosa Bay and Uranouchi Inlet, Kochi, Japan showing the sampling areas in black patches/circles.

RESULTS

Historical Conservation Status

Eight dragonet populations/species are found to be in stable conditions namely: *Callionymus planus* Ochiai, 1955, *Callionymus lunatus* Temminck and Schlegel, 1845, *Callionymus curvicornis* Valenciennes, 1837, *Callionymus*. *japonicas*, *Callionymus enneactis* Bleeker, 1879, *Synchiropus altivelis* (Temminck and Schlegel, 1845), *Repomucenus virgis* (Jordan and Fowler, 1903), and *Repomucenus huguenini* Bleeker, 1858. Others are globally endangered, locally vulnerable, locally highly vulnerable, and globally highly vulnerable (Table 3).

Genetic Variation and Species Identification

In terms of genetic diversity, *R. virgis* (18.0%) shows the highest percentage of polymorphism (P), while the lowest is *Callionymus beniteguri* Jordan and Snyder, 1900 (0.0%) (Figure 1). For heterozygosity (H), *C. planus* is the most diverse (15.6%), and *C. beniteguri* (0.0%) is the lowest. For the

Table 3. Qualitative categories for the conservation status based on the arbitrary scores of the reported dragonet populations in Tosa Bay. P-polymorphism; H-heterozygosity. *recently not collected in Tosa Bay; **no data.

Species	Genetic variation		Abundance	Depth distribution	Geographic range	Frequency	Conservation status (total=12)
	P	H					
Bathycallionymus kaianus	ave. (1)	ave. (1)	rare (0)	restricted (0)	widespread (2)	scarce (0)	locally vulnerable (4)
Callionymus beniteguri	low (0)	low (0)	rare (0)	restricted (0)	domestic (1)	scarce (0)	locally highly vulnerable (1)
Callionymus curvicornis	ave. (1)	ave. (1)	ave. (1)	widespread (2)	domestic (1)	ubiquitous (2)	stable (8)
*Callionymus draconis**	**	**	**	**	endemic (0)	**	globally endangered (0)
Callionymus enneactis	ave. (1)	ave. (1)	ave. (1)	restricted (0)	widespread (2)	ubiquitous (2)	stable (7)
Callionymus formosanus	ave. (1)	ave. (1)	rare (0)	restricted (0)	domestic (1)	scarce (0)	locally vulnerable (3)
Callionymus japonicas	ave. (1)	ave. (1)	abundant (2)	ave.(1)	widespread (2)	ubiquitous (2)	stable (9)
Callionymus lunatus	high (2)	high (2)	abundant (2)	widespread (2)	domestic (1)	ubiquitous (2)	stable (11)
Callionymus planus	ave. (1)	high (2)	ave. (1)	restricted (0)	endemic (0)	ubiquitous (2)	stable (6)
Callionymus sokonumeri	ave. (1)	low (0)	rare (0)	restricted (0)	endemic (0)	scarce (0)	globally highly vulnerable (1)
*Callionymus valenciennei**	**	**	**	**	domestic (1)	**	locally highly vulnerable (1)
Foetorepus masudai	**	**	rare (0)	**	endemic (0)	scarce (0)	Insufficiently known (0)
Repomucenus huguenini	ave. (1)	high (2)	abundant (2)	widespread (2)	domestic (1)	ubiquitous (2)	stable (10)
Repomucenus virgis	high. (2)	high (2)	abundant (2)	widespread (2)	domestic (1)	ubiquitous (2)	stable (11)
Synchiropus altivelis	ave. (1)	ave. (1)	abundant (2)	widespread (2)	domestic (1)	ave. (1)	stable (8)

frequency, *R. huguenini* and *Callionymus japonicus* Houttuyn, 1782 (15.9%) are the most frequent species caught, while *C. beniteguri* (0.9%) and *Callionymus sokonumeri* Kamohara, 1936 (0.0%) are the least frequent (Figure 1). In numerical abundance (NA), *R. huguenini* (68.6%) is the most abundant, followed by *C. japonicus* (9.2%), and *C. lunatus* (7.8%) (Figure 1).

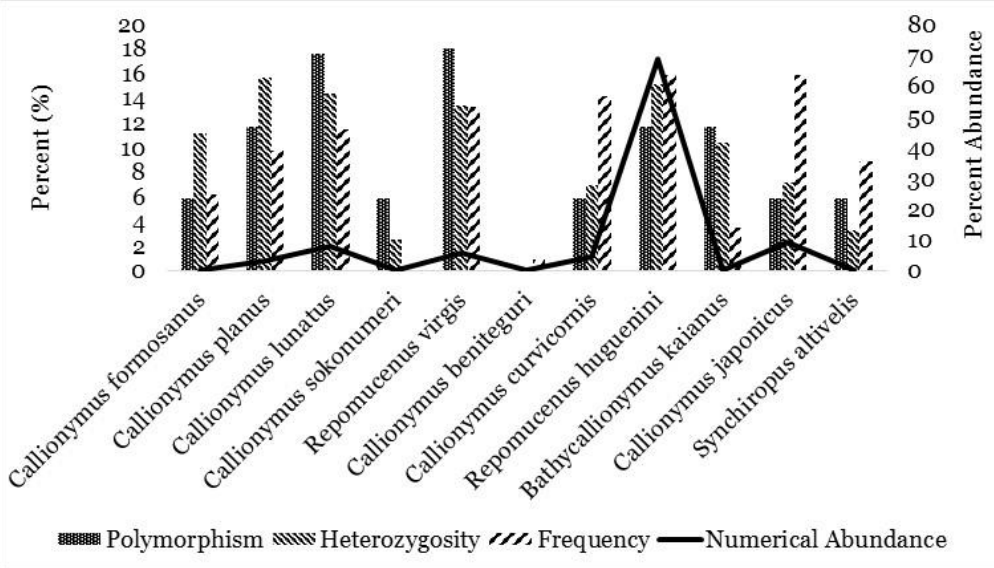

Figure 2. Genetic variation (proportion of Polymorphic (P) loci and mean Heterozygosity (H)), percent monthly Frequency (F) appearance, and with Numerical Abundance (NA) of 11 dragonets during the 18-month trawling in Tosa Bay (1993-1995).

The combination of an ecological and genetic set of indicators showed a significant result in measuring the conservation status of the identified population/species of dragonets in Tosa Bay and Uranouchi Inlet. Among the indicators used, Polymorphism (P) and Heterozygosity (H); Frequency (F) and Numerical Abundance (NA) were highly correlated. Heterozygosity (H) and Numerical Abundance (NA); Depth Distribution (DD) and Numerical Abundance (NA) were correlated (Table 4).

Table 4. The correlation coefficient of genetic (Polymorphism-P, Heterozygosity-H) and ecological (Numerical Abundance-NA, Frequency-F, Depth Distribution-DD, Geographic Range-GR) indicators, using a 2-tailed test (Spearman's rho). **highly significant, *significant.

Parameters	P	H	NA	F	DD	GR
P Correlation Coefficient	1.000	0.799**	0.501	0.367	0.383	0.000
Sig. (2-tailed)		0.003	0.117	0.267	0.245	1.000
H Correlation Coefficient	0.799**	1.000	0.655*	0.506	0.211	-0.095
Sig. (2-tailed)	0.003		0.029	0.113	0.533	0.780
NA Correlation Coefficient	0.501	0.655*	1.000	0.952**	0.663*	0.286
Sig. (2-tailed)	0.117	0.029		0.000	0.026	0.394
F Correlation Coefficient	0.367	0.506	0.952**	1.000	0.710*	0.311
Sig. (2-tailed)	0.267	0.113	0.000		0.014	0.353
DD Correlation Coefficient	0.383	0.211	0.663*	0.710*	1.000	0.158
Sig. (2-tailed)	0.245	0.533	0.026	0.014		0.642
GR Correlation Coefficient	0.000	-0.095	0.286	0.311	0.158	1.000
Sig. (2-tailed)	1.000	0.780	0.394	0.353	0.642	

DISCUSSION

Historical Conservation Status

There are varying degrees of conservation risks to the populations of *Callionymus valenciennei* Temminck and Schlegel, 1845, *C. beniteguri, C. draconis, C. sokonumeri, Callionymus Formosanus* Fricke, 1981, and *Bathycallionymus kaianus* (Günther, 1981). However, it is possible that because dragonets are not popular fish food and have low commercial value, people locating their samples may fail to record or report their existence in certain locations. Therefore, current and further exhaustive fieldwork must locate the undiscovered and unreported dragonet species.

Two previously reported species in Tosa Bay are not found in our samples – *C. valenciennei* and *C. draconis* (Table 3). The non-appearance of *C. valenciennei* and *C. draconis* in our samples indicates the possibility that their populations may not anymore exist in Tosa Bay, making it very tempting to declare them as locally extinct or presumed extinct species or populations. However, extinct species are those for which there is no doubt that the last member of the species has died; and presumed extinct species are those that have not been located in the wild for the last 50 years (Burgman et al. 1993). Thus, these conditions do not apply to the present status of *C. valenciennei* and *C. draconis*. On the other hand, an endangered species is defined as those facing a high risk of extinction within one or two decades, and vulnerable species are those not currently endangered but are at risk over longer periods (usually 50 to 100 years) if factors tending to push the species into decline continue to operate (Burgman et al. 1993). According to Fricke (pers. comm.), *C. draconis* is a very rare species, therefore difficult to collect and rarely reported, while *C. valenciennei* is more common, even commercially used in Japan, which is consistent with our results in Table 3 – *C. draconis* is globally endangered, only found in the Pacific Coast of southern Japan, while *C. valenciennei* is locally highly vulnerable in Tosa Bay. When something is rare, it is not necessarily threatened with imminent extinction, just as species that are likely to become extinct soon are not necessarily rare, restricted, or specialized (Burgman et al. 1993). Rare, restricted, and specialized species are not presently vulnerable and may be present in stable populations, but some characteristics of their population sizes or distributions make them conceivably at risk in the long term (Burgman et al. 1993). Hence, although the population of *C. draconis* in Tosa Bay is very rare (Nakabo 1977), it could be in stable condition.

Genetic Variation and Species Identification

The genetic variability values of *F. masudai* could not be obtained, because only one sample was collected, and our survey did not cover its entire depth distributional range, 120-400 m in Tosa Bay. Thus, we termed its conservation status as ‘insufficiently known” defined by Burgman et al. (1993) as those species with insufficient information on which to base a judgment concerning either their abundance and distribution or the degree of threat they face. *Callionymus beniteguri* is locally highly vulnerable. The values for genetic variation, numerical abundance, and frequency of occurrence in Tosa Bay (Table 3 and Figure 1), show that *C. curvicornis* and *R. huguenini* are dominant and widely distributed species in the area. The rare *C. beniteguri* occurs sympatrically with its four widely distributed congeners like *R. huguenini*, *C. lunatus*, *C. planus*, and *C. curvicornis* in the shallow waters of Tosa Bay, which have similar dietary resource requirements (Gonzales et al. 1996a), co-occurring spawning seasons (Eda et al. 1994; Gonzales and Taniguchi 1997b) and similar spawning behavior (Gonzales et al. 1996b). Furthermore, some dragonets have territorial behavior, and fighting among males occur during spawning (Takita and Okamoto 1979; Gonzales et al. 1996b). These similarities in the basic biological and ecological requirements (e.g. in reproductive resources, in prey organisms) of the four species indicate close competition among them, which most likely have resulted in the decrease in reproductive fitness of *C. beniteguri* in Tosa Bay.

The survival rate of 40-days juveniles of *C. curvicornis* and *C. valenciennei* is 7-8 times greater than *C. beniteguri*. Although *C. beniteguri* has a larger length-sized newly hatched pro-larvae, it has a relatively smaller forty-day-old juvenile than *C. curvicornis* and *C. valenciennei* (Eda et al. 1994), which show relatively low survival and growth rates in the early life-stages of *C. beniteguri*. This loss in the early life fitness of *C. beniteguri* in Tosa Bay may be an effect of genetic drift or inbreeding in its low-density population, as observed by Meffe (1990) in other fishes. The situation might have been further aggravated when the individuals in the already small population presumably inbred, likely expressing deleterious recessive alleles in the population (Meffe 1990), subsequently resulting in the loss in early-life fitness of the population. Anthropogenic effects may also be a probable factor, as dragonets were often observed caught as by-catch species during fishing operations.

Additionally, the genetic dendrogram of Gonzales et al. (1997a) shows that *C. beniteguri* is a fairly newly-evolved species. The more recently derived rather than the oldest members of a community that show evidence of interspecific interactions that could lead to competition-mediated extinction (Brooks et al. 1992). Hence, the natural phenomena on the speciation and extinction cycle could not be a driver for the rarity of *C. beniteguri*. In summary, the cause of the decline of the population of *C. beniteguri* in Tosa Bay could be its competition for reproductive and food resources against the widely distributed *R. huguenini*, *C. lunatus*, and *C. curvicornis*, and partly by human actions.

The geographic distribution of *C. sokonumeri* shows that its extinction in Tosa Bay may mean extinction in its whole geographic range. Hence immediate, more detailed investigation on the causes of its rarity and subsequent management is highly recommended. *Callionymus sokonumeri* is endemic in the Pacific coast of southern Japan, and its present habitat shows that it could be a resident species in that area, presumably occurring within the dispersal area of dragonets (90-120 m) Pacific coast of southern Japan as supported by Gonzales and Taniguchi (1997b). Those species that have been part of any given biota for the longest periods maybe are most in need of protection against exploitation and removal (Brooks et al. 1992). Thus, the management of *C. sokonumeri* must include the conservation of its area of endemism (Pacific coast of southern Japan), though there is much to know about their microhabitat. *Callionymus planus* and *C. draconis* are also known to be endemic in the same area. While, *C. formosanus*, *C. sokonumeri*, and *B. kaianus* have no genetic information, and its available data is only ecological –depth and geographic distribution. This constrains the making of inferences on the rarity of the above species, though the rarity of *C. formosanus* is highlighted by Gonzales and Okamura (1995).

Scientists and researchers use different strategies to provide scientific approaches to protect biodiversity. Some workers utilize ecology-based indicators for biodiversity assessment (Jennings 2005, Siddig et al. 2016; Wendling et al. 2018), while genetic indicators approaches are popular to others (e.g. Meffe 1990; Ashbaugh et al. 1994; and Primack 1995). However, to monitor biodiversity loss at the global, regional, and local levels, a wealth of indicators was created over the last two decades, but genetic diversity indicators are regrettably absent from a comprehensive bio-monitoring scheme (Graudal et al. 2014). Hence, the use of both ecological and genetic indicators may not be common and must be promoted to biodiversity workers.

In support to the above argument, this study shows that combining ecological and genetic indicators has successfully determined the status of different populations and species of closely associated fishes in a management unit and provides information on their conservation status and priorities. This study provides historical baseline information on the two-decade conservation status of dragonets in the Bay; hence, it is important to conduct follow-up studies to compare the before and after changes and trends of the fish species/population status in the Bay.

Using two or more parameters increases the coverage of an assessment, thereby measuring more array of characters and traits, and cross-checking possible bias that may be brought about when using only indicators from one parameter–ecological or genetic. In *C. planus*, for example, vulnerable status was revealed when only ecological indicators were used, but when combined with genetic variation indicators, the population turn out to be in stable condition (Table 3). The temporal aspect of ecological and genetic evolutions is an interesting topic to further the inferences when combining biodiversity indicators from different parameters.

The conservation assessment (Table 3) showed that varying degrees of conservation status could be measured using quantitative categories converted into qualitative ranges. This is a viable tool in population risk assessment that also provides some scientifically-based options in management policies for the preservation of biodiversity in a defined spatial management unit–like southwestern Japan.

This conservation status measurement approach is suitable to any local government or entity managing a spatial management unit, e.g., marine protected areas, key biodiversity areas, parks, sanctuaries, bays, islands, lakes, and any area with high biodiversity value. This approach may not yet be perfect and have much space for improvement, but it does provide a substantial point for sound decision-making based on hard science.

Since the conservation status of the populations in this study was measured based on the 1990s survey, it could serve as a historical baseline for future similar or follow-up studies in Tosa Bay or may be used to assess other species and populations. The Pacific coast of Southern Japan should also be further investigated for possibly more endemic species. The subsequent management policies for the species must include not only the preservation of populations, but also the protection of habitats, especially endemism area. On the other hand, social and governance aspects of conservation shall be closely considered in the final development of the species conservation plan and a specific conservation program shall be developed for *C. planus* and *F. masudai*.

ACKNOWLEDGMENTS

This study is funded by the Monbushio Fellowship Program of the Ministry of Education and Culture of the Government of Japan. We thank the evaluators for improving the early versions of this paper. Thanks are due to my laboratory mates, and the captain and crew of Toyohata Maru for the field assistance. We thank Drs. Ronald Fricke and Tetsuji Nakabo for providing valuable references and information. We are grateful to Dr. Osamu Okamura and Dr. Masao Ono for the suggestions and use of their laboratories. And to Dr. Shingo Seki for his advice and assistance in the electrophoresis experiments. Thanks are due to Niño Jess Mar F. Mecha, Sharon Rose Anunciado and Clarilyn Lariza for their assistance to this paper.

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ARTICLE INFO:

Received: 15 May 2020
Revised: 15 July 2021
Accepted: 07 August 2021
Available online: 24 August 2021

Role of authors: BJG – conceptualized the study, collected, processed and analyzed the data, and wrote the paper; NT – supervised the first author, co-conceptualized the study, provided insights to the discussion, sourced out funds, facilitated field works, and reviewed the manuscript.