

Rapid Communication**First record of the non-indigenous Indo-Pacific damselfish, *Neopomacentrus cyanomos* (Bleeker, 1856) in the northern Gulf of Mexico**Clayton T. Bennett^{1,2}, Alison Robertson^{1,2,*} and William F. Patterson III³¹Department of Marine Sciences, University of South Alabama, Mobile, Alabama, USA²Marine Environmental Sciences Consortium, Dauphin Island Sea Lab, Dauphin Island, Alabama, USA³University of Florida, Gainesville, Florida 32653, USAAuthor e-mails: cbennett@disl.org (CTB), arobertson@disl.org (AR), will.patterson@ufl.edu (WFP)

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Received: 18 February 2018**Accepted:** 14 August 2018**Published:** 15 October 2018**Handling editor:** Charles Martin**Thematic editor:** Amy Fowler**Copyright:** © Bennett et al.This is an open access article distributed under terms of the Creative Commons Attribution License ([Attribution 4.0 International - CC BY 4.0](https://creativecommons.org/licenses/by/4.0/)).**OPEN ACCESS****Abstract**

Diver and remotely operated vehicle surveys have produced the first record of the invasive regal demoiselle, *Neopomacentrus cyanomos*, from the northern Gulf of Mexico (GOM) in July 2017. Several hundred individuals were observed associated with petroleum platforms and artificial reefs off the coast of Alabama. Initial taxonomic identification was made via morphometrics, coloration patterns, and meristic counts, and species was subsequently confirmed via DNA barcoding of the mitochondrial cytochrome *c* oxidase gene. This new finding represents a significant range expansion of the Indo-Pacific species in the greater GOM. Prior to our finding, there had been no reports of the species outside the southwestern regions of the GOM. Collection of early-stage juvenile specimens along with large adults suggests that further expansion is likely. Potential ecological impacts of a non-indigenous damselfish invasion are currently unknown and difficult to predict. Further study on the life history and ecology of the *N. cyanomos* population in the GOM, including their interaction with the invasive Indo-Pacific lionfish (*Pterois volitans*), will provide much needed information for understanding potential impacts and for management and mitigation of this species.

Key words: regal demoiselle, DNA barcoding, range expansion, invasive species, marine invasion ecology

Introduction

Marine species invasions in the Gulf of Mexico (GOM) have been linked to increased shipping traffic (Benson et al. 2001; Hicks and Tunnel 1999; Lemaitre 1995), extensive oil and natural gas activities (Sheehy and Vik 2010), and aquarium or aquaculture releases (Fuller et al. 2014). Three exotic species from the Indo-Pacific Ocean introduced by these vectors have already become established in the greater GOM and Caribbean Sea, including the orange cup coral, *Tubastraea coccinea* (Lesson, 1829), giant tiger prawn, *Penaeus monodon* (Fabricius, 1798), and lionfish, *Pterois volitans/miles* (Linnaeus, 1758/Bennett, 1828) complex (Fenner 2001; Selwyn et al. 2017; Wakida-Kusunoki et al. 2013). Another non-indigenous Indo-Pacific fish, the regal demoiselle, *Neopomacentrus cyanomos* (Bleeker,

1856), was discovered inhabiting coral reefs off the coast of Veracruz, Mexico in 2013 (González-Gándara and de la Cruz-Francisco 2014) and, until recently, was thought to be restricted to the Bay of Campeche in the southwest GOM based on an ocean dispersal model and reef surveys in the greater GOM and Caribbean (Johnston and Akins 2016; Robertson et al. 2016b).

Neopomacentrus cyanomos is native to the Indian and western Pacific Oceans, ranging from the east coast of Africa near Madagascar, north to the Red Sea, east across the Asian coast to the Philippines and southern Japan, and south through Indonesia and Melanesia to the northern coast of Australia (Allen 1991). In its native range, *N. cyanomos* lives associated with coral reefs at depths to 25 m (Allen 1991) where it forms large aggregations while feeding on suspended plankton near the reef. These fish are likely monomorphic protogynous hermaphrodites (although not yet confirmed by laboratory studies) (Sreeraj and Gopakumar 2004), with smaller individuals being females that transition to males during maturation or after a decrease in the number of males. Breeding at lower salinities (22–24 psu) has been demonstrated in aquaria, which implies a physiological tolerance to salinity below full strength seawater (35 psu), but the lower salinity limit for this species is unknown (Setu et al. 2010). In terms of reproduction, like most pomacentrids, a single *N. cyanomos* male will clear algae and debris from a portion of substrate and then allow a female to deposit a clutch of adhesive eggs for fertilization (Loh et al. 2013; Setu et al. 2010). The male provides subsequent care throughout the incubation period by removing debris and any compromised eggs, while guarding against predation (Loh et al. 2013; Setu et al. 2010). A male will subsequently fertilize the eggs of multiple females at a single nest site, and females can spawn multiple times during a season (Loh et al. 2013). Under laboratory conditions *N. cyanomos* have been reported to hatch 3–5 days post-fertilization (Loh et al. 2013; Rohini Krishna et al. 2016; Setu et al. 2010). Field studies have shown that hatched larvae then enter a pelagic planktonic stage for 17–18 days before recruiting to reef structure (Thresher et al. 1989).

The regal damselfish was identified in June 2013 in the southwest GOM on coral reefs near Coatzacoalcos, Mexico (González-Gándara and de la Cruz-Francisco 2014). Aggregations of 5–30 *N. cyanomos* were sighted on dive surveys during June–September 2013 from depths of 2–21 m, with higher site fidelity noted on deeper reefs (González-Gándara and de la Cruz-Francisco 2014). In September 2015, additional divers searched for *N. cyanomos* during surveys of coral reefs at Stetson Bank, as well as East and West Banks of the Flower Garden Banks Marine Sanctuary (Robertson et al. 2016b). Investigators also searched the Reef Environmental Education Foundation's (REEF) sighting database (<http://www.reef.org>) and United

States Geological Survey's Non-indigenous Aquatic Species (USGS NAS) database (<https://nas.er.usgs.gov>) in late September 2015 for possible diver reports of the species in the Florida Keys (Robertson et al. 2016b). At that time (late September 2015), *N. cyanomos* was not reported beyond the southern GOM sites by any of these methods (Robertson et al. 2016b). However, aggregations of up to 150 individuals were reported from Cayo Arcas, an offshore reef complex along the Campeche Bank in the southwest GOM in August 2016, and revision of photographs from July 2013 also confirmed presence of *N. cyanomos* at these sites near the time of the initial discovery at Coatzacoalcos (Robertson et al. 2016a). Since these findings in the southern GOM, no reports of *N. cyanomos* in the northern GOM have been made. Likewise, at the time of our first regal damselfish finding (July 2017), no local sightings were reported in the USGS NAS (<https://nas.er.usgs.gov>) or REEF (<http://www.reef.org>) invasive species databases, so it is currently unknown how and when *N. cyanomos* became established in the northern GOM.

In the present study, we report the first observation of *N. cyanomos* in the northern GOM, which represents a major shift in the geographic range of this species. Following preliminary identification, study objectives were to verify the presence of *N. cyanomos* via species-specific morphometrics and meristics, confirm species identification via DNA barcoding, and survey the north central GOM more broadly to determine the prevalence of *N. cyanomos* populations in the region.

Methods

Surveys were conducted by divers and with a remotely operated vehicle (ROV) at petroleum platforms, artificial reefs, and natural reefs over an approximately 13×103 km² area of the northern GOM shelf (longitude range: 85.5W to 88.25W; depth range: 11–82 m) from May through December 2017 (Figure 1, Supplementary material Table S1). A primary purpose of these surveys was to document the small demersal fish communities at study sites, including native damselfishes. Paired diver surveys were conducted for independent secondary confirmation to identify presence of *N. cyanomos* and describe interactions with other fish species. Platforms and artificial reefs were extensively inspected by following along the entirety of structural surfaces. Depth of encounter using a dive computer (Oceanic GEO 2.0; San Leandro, California, USA), location relative to structure, number of individual *N. cyanomos* in groups, and interspecific interactions were recorded on dive slates. Hydrographic data were collected onboard the vessel with a SonTek Handheld CastAway CTD (San Diego, CA, USA).

Video sampling with ROV was performed only at natural and artificial reefs. The ROV was a VideoRay Pro4 micro ROV (dimensions: 36 cm long,

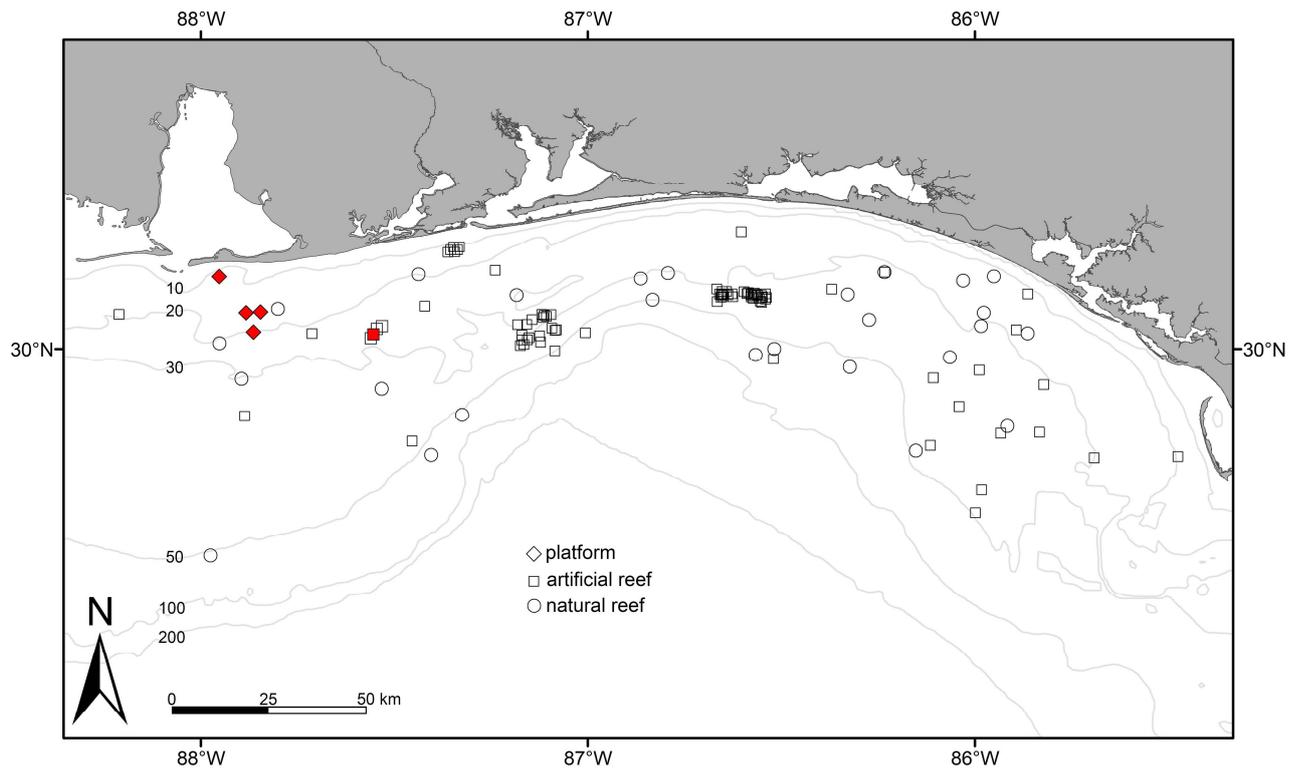


Figure 1. Distribution of field sites including petroleum platforms, natural reefs, and artificial reefs surveyed across the northern Gulf of Mexico from May to December 2017 by divers and a remotely operated vehicle. Open symbols indicate habitat type and filled symbols indicate locations of *Neopomacentrus cyanomos* observations (for details see Supplementary material Table S1).

28 cm tall, 22 cm wide; mass = 4.8 kg; Pottstown, PA, USA), which has a depth rating of 170 m and a 570-line color camera with wide angle (116°) lens. In addition, a 2.7k, 60 fps high-resolution digital camera with wide angle (128°) lens was deployed as a secondary camera on the ROV. The ROV was tethered to the surface where it was controlled by a pilot via an integrated control box with a 38-cm video monitor to observe ROV position during sampling. The point-count method described by Patterson et al. (2009) was utilized to sample small (< 50 m³) artificial reefs, while the transect method described in Dahl and Patterson (2014) was utilized to sample larger artificial reefs and all natural reefs.

Following initial surveys, sites where aggregations of *N. cyanomos* were noted were revisited by divers to sample fish. Fish identified as *N. cyanomos* during underwater surveys were anesthetized (~ 1 min) using a clove oil solution (20% clove oil and 80% ethanol) delivered within 20 cm of the fish with a 50-mL syringe. Fish were then captured with a small fine mesh net or pole spear with 3-prong paralyzer tip, and specimens were retained in a clear collection tube with neoprene closure at depth.

Fish identified visually as *N. cyanomos* were euthanized via ice water immersion and spinal cord dislocation according to approved IACUC protocols. Measurements including standard, fork, and total lengths to the nearest millimeter and mass to the nearest 0.01 gram were recorded. Taxonomic identification was performed using coloration, meristic, and

morphological characteristics described by Allen (1991). Juvenile specimens of *N. cyanomos* were compared to figures and morphometric data described in Rohini Krishna et al. (2016). Following initial species identification, a subset of samples ($n = 13$) was selected for DNA barcoding to confirm species identification across the size range. Whole genomic DNA was extracted using a QIAGEN DNeasy Blood and Tissue Kit following established protocols. Muscle tissue (approximately 50 mg) was removed from skin using flame sterilized forceps and scalpel, placed in sterile 1.5 ml micro-centrifuge tubes, and stored at $-80\text{ }^{\circ}\text{C}$ until extraction. Tissue was homogenized in tissue lysis buffer and proteinase k solution then lysed by incubation at $56\text{ }^{\circ}\text{C}$ for approximately 2 hrs. The isolation and elution steps were followed as per the standard protocol with a second elution in AE buffer to maximize total DNA yield. DNA was quantified using a NanoDrop 1000 Spectrophotometer (Thermo, Wilmington, DE, USA) and maintained at $-80\text{ }^{\circ}\text{C}$ until polymerase chain reaction (PCR) was performed. DNA concentrations ranged from $13\text{--}58\text{ ng ul}^{-1}$ and produced a 260/280 nm absorbance ratio of approximately 2.2.

Amplification was performed for the 650 base pair (bp) region of cytochrome *c* oxidase-1 (COI) mitochondrial gene using 5' end M13 tailed primers VF2_T1, FishF2_T1, FishR2_T1, FR1d_T1 recommended for barcoding of teleost fish (Ivanova et al. 2007; Ward et al. 2005) and used in previous studies of invasive species (Dahl et al. 2017). The PCR mix was derived from methods described by Ivanova et al. (2007), and each 50 μl reaction consisted of 25 μl of 0.26 M trehalose dihydrate (MP Biomedicals, CA; prepared in nuclease-free water), 8.5 μl PCR-grade water (Millipore Sigma, MA), 5 μl of 10X PCR buffer, 5 μl of 25 mM MgCl_2 , 0.25 μl of 10 mM dNTP mix (Applied Biosystems, CA), 0.5 μl of each 10 μM primer (custom oligos prepared by Millipore Sigma, MA), 1.25U of Taq DNA polymerase (Fisher Scientific, NH), and 4 μl of DNA template extracted from northern GOM fish specimens. Reagent purity was tested with negative controls where template was substituted with nuclease free water (Invitrogen, CA). A Bio-Rad C1000 thermal cycler (Hercules, CA, USA) was used for amplification, and parameters included an initial denaturation step at $95\text{ }^{\circ}\text{C}$ for 2 min followed by 35 cycles of $94\text{ }^{\circ}\text{C}$ for 40 sec (denaturation), $53.5\text{ }^{\circ}\text{C}$ for 40 sec (annealing), and $72\text{ }^{\circ}\text{C}$ for 1 min (extension), and a final extension at $72\text{ }^{\circ}\text{C}$ for 5 min. Following the PCR, samples were held at $4\text{ }^{\circ}\text{C}$, and products were subsequently visualized on 1.2% agarose gels with a 100 bp ladder (Millipore Sigma, MA). Amplicons were submitted to GENEWIZ[®] (South Plainfield, NJ, USA) for final purification and bidirectional Sanger sequencing using universal M13F and M13R primers.

The mitochondrial COI amplicons were processed and analyzed using Geneious (v11.0.4; Biomatters Ltd. New Zealand) and subsequently compared to a suite of nucleotide databases including GenBank, the European

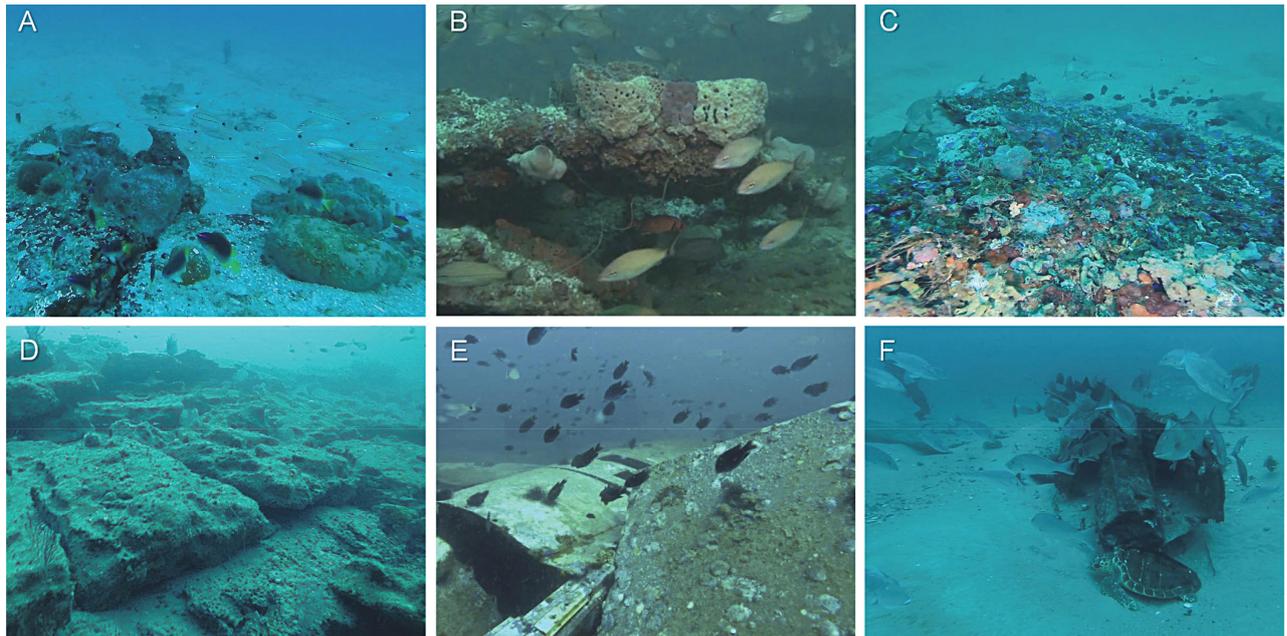


Figure 2. Still images captured from remotely operated vehicle digital video (2.7k resolution and 60 fps) samples of northern Gulf of Mexico natural (A–D) and artificial (E–F) reef sites sampled in summer and fall 2017. Sites A–C are limestone reefs with attached sponges and gorgonians and are in 20–40 m depths off Pensacola and Destin, Florida. Site D is a mesophotic sandstone reef in 80 m depth southeast of Destin, Florida. Site E is a bridge rubble artificial reef at 32 m south of Pensacola, Florida, while site F is a sunken airplane artificial reef in 41 m depth south of Destin, Florida. Native damselfishes were observed at sites A (yellowtail reeffish, *Chromis enchrysur* and juvenile beaugregory, *Stegastes leucostictus*), C (purple reeffish, *Chromis scotti*, blue chromis, *Chromis cyanae*, cocoa damselfish, *Stegastes variabilis*, and beaugregory), and E (purple reeffish and cocoa damselfish). Photographs by Joe Tarniecki.

Molecular Biology Laboratory nucleotide archive, Research Collaborative for Structural Bioinformatics, and the NCBI Reference Sequence Database. Raw chromatograms were visually inspected, edited, and trimmed to remove primers and poor-quality reads. Sequence alignments were performed on forward and reverse sequences with a 93% similarity matrix, gap open penalty of 15, and a gap extension penalty of 5 to produce a high-quality consensus sequence. The basic local alignment search tool (BLAST) was used to compare the final consensus sequences with target nucleotide databases (e.g., GenBank) to determine statistically significant local alignments. Parameters, including the E value (number of expected matches by chance relative to database size), percent pairwise identity (% similarity between two sequences), and grade (% combining E value, percent overlap of query/subject sequences, and % pairwise ID), were used to determine the highest probability matches of voucher specimen sequences contained in the database. Sequences > 390 base pairs and graded > 99% with vouchers were confirmed as *N. cyanomos*. These criteria for species level identification follow the conservative values proposed by Dahl et al. (2017).

Results

Diver or ROV surveys were conducted at 138 reef sites in the northern GOM during summer and fall 2017, which included petroleum platforms and natural and artificial reefs (Figure 1). Natural reefs, which consisted of either relict limestone or sedimentary sandstone structures (Figure 2),

tended to be more structurally complex than artificial reefs, although petroleum platforms had the greatest relief. Several hundred *N. cyanomos*, including hundreds of juveniles in groups of 10–35, were observed on five petroleum platforms and one artificial reef (i.e., a concrete cylinder approximately 7.5 m tall and 1.5 m wide in 29 m of water surrounded by sand). No *N. cyanomos* were observed on artificial reefs < 7.5 m in height or on any natural reefs in the region, and no *N. cyanomos* were observed during ROV sampling. However, native damselfishes species, including *Chromis cyanea* (Poey, 1860), *Chromis enchrysur* (Jordan and Gilbert, 1882), *Chromis scotti* (Emery, 1968), *Stegastes leucostictus* (Muller and Troschel, 1848), and *Stegastes variabilis* (Castelnau, 1855), including juveniles, were routinely observed in ROV video samples (Figure 2).

Among the petroleum platform sites, water temperature ranged from 29.3 °C at surface to 27.2 °C on the bottom, with the pycnocline occurring at approximately 10.7 m. Encrusting invertebrates (e.g. barnacles, bryozoans, and sponges) and macroalgae characterized the platform substrate. Some aggregations of *N. cyanomos* were observed on petroleum platforms in close proximity to native cocoa damselfish, *S. variabilis*, a solitary benthic omnivore that displays territorial behavior. Adult *S. variabilis* displayed antagonistic behavior toward *N. cyanomos*, attempting to push them away from the structure. Another observation included *N. cyanomos* clinging inverted to the underside of a flat surface of the rig structure at 20 m, a cryptic behavior noted in other studies (e.g., Robertson et al. 2016b). An important note during diver surveys was the presence of an invasive lionfish, *P. volitans* (approximately 25 cm TL), which was actively consuming an individual *N. cyanomos* at one rig site.

Neopomacentrus cyanomos observed and collected during this study had a body coloration that was typically dark brown to black with somewhat lighter shading along the ventral half of the fish. Soft dorsal and caudal fins had yellow or white markings, but were highly variable, consistent with previous reports (Allen 1991). A yellow or white spot at the posterior base of the dorsal fin and a black ear spot slightly superior to the eye and overlapping the posterior edge of the operculum distinguished this fish from other native damselfishes (Figure 3). The caudal fin was forked with a slightly longer upper lobe and yellowish coloration in the center of the tail. Filamentous trailing edges of the soft rays were present from all fins except the pectorals, and the ventral side of the body and edges of the ventral and dorsal fins were speckled with brilliant blue or white in fresh specimens. The suborbital was covered by scales. The following meristics were consistent with Allen (1991): dorsal fin XIII (11–12); anal fin II (11–12); pectoral fin (17–18); lateral line scales 17–18.

All 42 damselfish captured from field sites in the northern GOM (see Figure 1) were identified as *N. cyanomos* based on coloration, meristic counts,



Figure 3. Representative *Neopomacentrus cyanomos* (A: 45 mm Standard length (SL); B: 13 mm SL) collected from the northern Gulf of Mexico in Summer 2017. This species is discernible from other damselfishes by a dark ear spot below the first dorsal spine and white/ yellow coloration on the bottom of the soft dorsal and center of the caudal fin that appear white underwater. Scale is common to both images. Photographs by (A) Brian Jones and Benjamin Brenner; (B) C. Bennett.

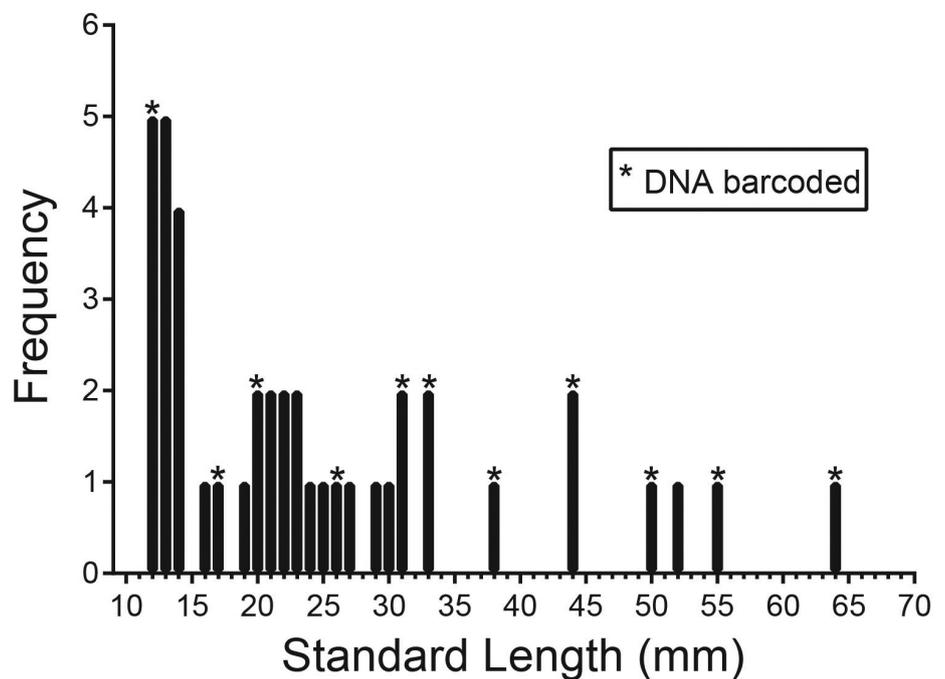


Figure 4. Frequency size distribution of *Neopomacentrus cyanomos* (n = 42) sampled in the northern Gulf of Mexico in 2017. All fish were identified as *N. cyanomos* (standard length = 12 mm to 64 mm) based on meristics and morphometrics. Asterisks indicate length bins for which individual fish were subsequently confirmed as *N. cyanomos* via DNA barcoding.

and morphometrics (e.g., Figure 3). These fish ranged between 12 and 64 mm SL (Figure 4). DNA barcoding of the mitochondrial CO1 region in the 13 randomly selected individuals confirmed species identity as *N. cyanomos* with

Table 1. Database matches to cytochrome *c* oxidase-1 nucleotide sequences of a subset of fish identified as *Neopomacentrus cyanomos* collected from the northern Gulf of Mexico in Summer 2017. Sequences > 390 bp and grade > 99% from collected specimens were positively verified to species. The expected value (E-value) for chance matches based on sequence and database size in all cases was 0. Contiguous sequences were deposited to GenBank (for accession numbers see Table S2).

Specimen ID	Length (bp)	Grade (%)	Similarity (%)	Top NCBI Match	Origin of Match	Matched Database Record
DISL_Regal 001	530	99.2	98.5	<i>N. cyanomos</i>	NE Australia	KP194522; KP193991
DISL_Regal 002	535	99.3	98.7	<i>N. cyanomos</i>	N Madagascar	JQ350144; JF435057
DISL_Regal 011	544	99.8	99.6	<i>N. cyanomos</i>	NW India	KF268144
DISL_Regal 018	533	99	99.8	<i>N. cyanomos</i>	NW India	KF268145; KF268146
DISL_Regal 020	544	99.5	99.6	<i>N. cyanomos</i>	NW India	KF268145
DISL_Regal 022	420	99.4	98.8	<i>N. cyanomos</i>	N Madagascar	JQ350144; JF435057
DISL_Regal 025	530	99.9	100	<i>N. cyanomos</i>	SW Gulf of Mexico	KU052527; KU052528
DISL_Regal 029	398	100	100	<i>N. cyanomos</i>	NW India	KF268145
DISL_Regal 030	430	99.8	99.5	<i>N. cyanomos</i>	NW India	KF268145
DISL_Regal 031	623	99.5	99	<i>N. cyanomos</i>	N Madagascar	JQ350144; JF435057
DISL_Regal 036	391	100	100	<i>N. cyanomos</i>	NW India	KF268145
DISL_Regal 038	544	99	99.8	<i>N. cyanomos</i>	NW India	KF268145; KF268146
DISL_Regal 039	444	100	100	<i>N. cyanomos</i>	NW India	KF268145

high probability (grade > 99%; E-value 0 in all cases; see Figure 4, Table 1). In all cases, the top 10 results for each barcoded fish specimen confirmed *N. cyanomos* vouchers and isolates from NCBI. Top voucher records were reported.

Discussion

The discovery of *N. cyanomos* inhabiting natural gas platforms and reefs in the northern GOM during July 2017 represents the first record of this invasive damselfish in the northern GOM. Although fish density was not quantified, the presence of relatively few large individuals (SL > 60 mm) on structures suggests the likelihood of a recent introduction. Due to the observed high abundance of juvenile individuals, successful colonization of the rest of the northern and eastern GOM is likely, if it has not already occurred. Since the time of our initial discovery in July 2017, other researchers and citizen-scientists across the Florida panhandle, which were notified of our discovery, have reported multiple records of *N. cyanomos* to the online USGS NAS (<https://nas.er.usgs.gov>), providing anecdotal evidence of broader species dispersal than the surveyed region. Further cross regional collections allowing meristic and, or, DNA confirmation would be useful to determine establishment success in this species. Additional observations from our laboratory in April 2018 have confirmed large adult *N. cyanomos* in the northern GOM have survived the winter at the sites previously surveyed suggesting tolerance to northern GOM salinity and temperature ranges.

González-Gándara and de la Cruz-Francisco (2014) suggested that *N. cyanomos* had likely been transported to the southern GOM in ship ballast water from the Indo-Pacific to the busy port at Coatzacoalcas, Mexico. This port and the Port of Tampico, Mexico have established trade

with the Port of Mobile, Alabama, the 10th largest port in the United States based on gross tonnage (AAPA 2016), and the northern GOM sites where *N. cyanomos* were first observed are approximately 5 km east of the Port of Mobile shipping lane. However, the possibility remains that early life stages of *N. cyanomos* were naturally transported from the southern to the northern GOM via the Loop Current (Kitchens et al. 2017; Lamkin 1997).

Artificial reefs and oil and gas platforms have been hypothesized to serve as corridors for non-indigenous species expansion, with more than 3,000 platforms currently distributed on the northern GOM shelf from Texas to Alabama (Sheehy and Vik 2010). Platforms extend substrate through the euphotic zone in deep water areas typically unsuitable for reef fish settlement and provide appropriate habitat for reef associated species (Lindquist et al. 2005). A report of thousands of *N. cyanomos* on an oil platform near Cayo Arcas in 2016 (Robertson et al. 2016a) coupled with our 2017 finding of the species on rig platforms in the northern GOM provides evidence that the structures supply suitable habitat. Also, this species has been shown to enter a nektonic stage after hatching and must settle on hard substrate in order to survive (Leis and Carson-Ewart 2003). The presence of petroleum platforms in areas of the northern GOM shelf without significant natural reef habitat may provide settlement and adult habitat from which propagules could disperse in the northern GOM (Kitchens et al. 2017; Sheehy and Vik 2010).

While aggregations of *N. cyanomos* on northern GOM petroleum platforms and artificial reefs have been confirmed during this study, further surveys will be required to determine whether *N. cyanomos* is able to establish long term populations. This is particularly important after winter since water temperatures drop significantly (from 29–30 °C to approximately 16 °C in winter). In prior studies, a temperature shift of just 6 °C, i.e., from 29 °C to 23 °C, reduced the metabolic and swimming performance of *N. cyanomos* (Johansen et al. 2015). However, the varied structure of petroleum platforms could allow physical relief from water column foraging, as has been proposed in other studies (Johansen et al. 2008; Johansen et al. 2015), and facilitate overwintering of *N. cyanomos*. Overwintering of coral reef damselfishes transported by the East Australia Current to temperate southeastern Australia, where water temperatures are comparable to the northern Gulf of Mexico (~ 17 °C), has also been reported and is another example of pomacentrid robustness (Figueira and Booth 2010). Where possible we suggest that prior year diver and ROV footage be reviewed to improve spatiotemporal knowledge of the *N. cyanomos* range expansion to the northern GOM. For instance, prior surveys and sightings database searches conducted in late September 2015 (Robertson et al. 2016b) did not find *N. cyanomos* in the Flower Garden Banks or Florida Keys. However, re-review of prior survey footage (or newly available

data) from other regions may help piece together migration history to the northern GOM over the past two years. Due to the cryptic nature of *N. cyanomos* and habitat preference for structure, these kinds of analyses may provide additional sightings records.

In the present study (as in others), DNA barcoding confirmation of taxonomic identity proved highly useful for *N. cyanomos* adults and early-life stages (approximately 30 days or less) which have under-developed features that may appear cryptic (Kitchens et al. 2017). Divers may not recognize inconspicuous species, particularly when an organism is observed in a non-indigenous area, so incorporation of this methodology greatly improved reliability of observational identification. This study represents the first report of an invasive regal damselfish *N. cyanomos* inhabiting gas platforms and artificial reefs in the northern GOM. It is still unclear what effects invasive damselfish will have on GOM reefs, native damselfishes, and potential predators including lionfish, all of which require further study in the northern GOM. Future research on *N. cyanomos* in the GOM should focus on the vectorization and ecological implications of this invasion to provide insight to the management of current and future invasions to the GOM.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Details of 2017–2018 distribution surveys conducted for *Neopomacentrus cyanomos* in the northern Gulf of Mexico (Alabama and northwest Florida).

Table S2. GenBank sequence accession numbers for *Neopomacentrus cyanomos* collected in 2017 from the northern Gulf of Mexico (Alabama).

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