Risk Assessment Model Development for Establishment Success and Impact of Non-native Freshwater Fishes in the Wet Tropics Bioregion, northern Queensland Australia

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A Report to the Marine and Tropical Science Research Facility (MTSRF) and Terrain Pty. Ltd.

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I EXECUTIVE SUMMARY

The study applied a risk assessment model for non-native freshwater fishes devised by Kolar and Lodge (2002) based upon previous invasion history to determine key species' attributes that may predict future outcomes of introductions in northern Queensland fresh waters. Thirty-two non-native species have been reported from Queensland, with 15 species having established populations, while 22 species have been reported from tropical northern Queensland with 11 species having established populations. Available data on the status (establishment and impact), ecology, life history and previous invasion history were collected from the literature for all species reported from Queensland fresh waters. For species where information was not available and fish were locally available, physiological tolerance trials were conducted to obtain maximum and minimum temperature, maximum salinity and minimum dissolved oxygen tolerance data. A total of 17 continuous and categorical variables were used (5 environmental tolerances, 6 life history attributes, 2 habitat needs, 3 previous invasion history variables, 1 phylogenetic similarity variable) for model development. Univariate (ANOVA) and multivariate analyses (Discriminant Analysis[DA], Categorical Regression Tree Analysis [CART] were performed on data sets for non-native fishes reported from Queensland and separately for northern Queensland waters. Analyses were done for the establishment and impact stages as defined by Kolar and Lodge (2002), using either the complete set of variables or a smaller set of directly-measured continuous variables (temperature, salinity and hypoxia tolerances, egg diameter and body size).

Species attributes obtained from statistical analyses that were strong predictors of establishment success or impact were similar for Queensland and for northern Queensland data sets, although classification accuracy was much lower for the impact stage analyses because of the small sample size available. The highest classification accuracy for the DA analysis was obtained for the larger Queensland data set (establishment stage: 92%). The highest classification accuracy for CART analysis was obtained for the larger Queensland data set and use of continuous, directly-measured variables only (establishment stage: 88.9%; impact stage: 73.3%). For construction of decision trees, the key predictors for establishment were critical minimum respiratory threshold, critical minimum and maximum temperature tolerances and egg diameter. The percentage of previously successful international introductions and number of human uses were the only two variables that were not ecological attributes and useful predictors of establishment success. For distinguishing between 'nuisance' and 'non-nuisance' species, the key predictors were critical temperature tolerance range, salinity tolerance, critical minimum respiratory threshold and egg diameter.

The results demonstrate that the Kolar and Lodge model, based on invasion history of non-native fishes in tropical and subtropical regions of Queensland and using predominantly ecological attributes, can provide a high level of classification accuracy for establishment and impact of these species in the Wet Tropics Bioregion. The Kolar and Lodge model identified species' attributes that are strong predictors of establishment success and impact, and improved classification accuracy by inclusion of directly measured ecological variables, especially for physiological tolerances, and provides a

more reliable assessment of a species' potential introduction compared with deductive models, especially where there is no previous invasion history.

II ACKNOWLEDGMENTS

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1.0 INTRODUCTION

Invasive species, including fishes, are now recognised as a major threat to global biodiversity (Witte *et al.* 1992; Cambray 2003; Njiru *et al.* 2005; Dudgeon *et al.* 2005) and can cause significant economic damage (Perrings *et al.* 2000; Pimental 2002; Cox & Kitchell 2004). Webb (2003, 2008) documented the history of introductions of non-native species into Australian waters and especially into tropical northern Queensland. Of concern was that in recent decades the rate of introductions appears to be accelerating. Historically, many introductions of non-native fish species, for example, for developing commercial fisheries and for the aquarium trade have occurred with little or no rigorous assessment of the risk these species may have posed to native aquatic communities if they entered open waterways and established feral populations. The increasing recognition of such threats resulted in the development of risk assessment protocols in several countries including Australia to regulate the import and release of non-native fishes, although such protocols were largely qualitative and limited due to lack of ecological information on target species. Kolar and Lodge (2004) noted that the screening method most often used for aquatic species involved deductive risk assessment, where inferences about a risk are based on what is known or has already occurred. This method is based on identification of species with a history of invasion and use of selection criteria to identify those with a higher potential of becoming invasive at a given location. They argued that this method is not based strictly on species characteristics, although attributes may be included in the selection criteria, usually in the form of subjectively ranked scores to determine category membership.

Deductive risk assessment has been used in New Zealand to identify potentially invasive species including aquatic weeds (Champion and Clayton, 2001) and in Australia for vertebrates including fish (Arthington et al., 1999; Harrison and Congdon, 2002; Hayes and Sliwa, 2003; Bomford and Glover, 2004; Bomford 2007). According to Koehn and MacKenzie (2004) assessments continue to be limited by a lack of knowledge of the biology and ecology of these species, not only in relation to their ability to establish, but also to their impacts on native communities. Kolar and Lodge (2004) pointed out that a weakness in using the deductive risk assessment approach is the assumption that all potential invaders have already invaded elsewhere. Invading species in an ecosystem sometimes have a very limited history or no history of previous invasion. In Australia, for example, the established noxious cichlid, *Tilapia mariae,* has had two previous introductions with only one being successful, and the established *Amphilophus citrinellus* has had five previous international introductions and only two being successful, while *Heros severus* has had two previous international introductions but did not establish. Two other cichlid species, *Haplochromis burtoni* and *Hemichromis lifalili,* with established populations in northern Queensland, have no previous invasion history (Webb, 2003, Webb, 2008) and the impacts of these species are unknown. Overseas, a notable example of a species introduction without previous invasion history was that of the lamprey, *Petromyzon marinus,* in the Laurentian Great Lakes, the species subsequently causing major economic and ecological impacts in the region (Mills et al., 2003; Bryan et al., 2005).

Kolar and Lodge (2004) stated that the next generation of risk assessment protocols need to be based on rigorous quantitative statistics rather than procedures that have historically relied largely on expert opinion or qualitative assessments. Such screening tools need to be transparent, relatively easy to use, reliable and unbiased (i.e., give the same results independent of user). They argued that this can be achieved using an approach based predominantly on life history characteristics and environmental tolerances of previous invaders in an ecosystem. This approach assumes that highly invasive species differ from unsuccessful species and, if they can be accurately distinguished from each other, then which species is likely to be highly invasive can be predicted. The approach recommended by Kolar and Lodge (2004) has now been used for model development to identify key invasive attributes of vertebrates including freshwater fish (Kolar and Lodge, 2002; Marchetti et al., 2004; Alcarez, 2005; Ruesink, 2005; Bomford and Glover, 2004; Jeschke and Strayer, 2006), which ranged in scale from regional to continental and global analyses. None of these studies were specifically for tropical/subtropical regions and each used different combinations and proportions of quantitative (continuous) and qualitative (categorical) variables. A range of attributes were identified as key predictors of invasion success with some similarities, but also differences across studies and with differing levels of classification accuracy.

Ruesink (2005), for a global analysis of vertebrates, found small body size, omnivorous diet, high endemisim in recipient country, and human use were the strongest predictors of invasion success. Marchetti et al. (2004) found measures of prior invasion success and propagule pressure were the strongest predictors for non-native fishes in California along with parental care, physiological tolerances and size of native range. Jeschke and Strayer (2006) found for North America and Europe, that measures of propagule pressure and human use were the strongest predictors. Kolar and Lodge (2002), for non-native fishes in the Laurentian Great Lakes region, North America, found different suites of characters were strong predictors when different multivariate statistical analyses of their data were performed. They found that rapid growth, wide salinity and temperature tolerances, and previous history of establishment using discriminant function analysis (DA) were key predictors of establishment success, while categorical and regression tree (CART) analysis indicated that minimum temperature, diet breadth, and relative growth rate were the strongest predictors. Kolar and Lodge (2002) also found different suites of attributes characterised the spread stage (slow growth rate, lower upper temperature tolerance and wide temperature tolerance range) and impact stage (small egg size, wider salinity tolerance and low temperature threshold) of the invasion process.

Webb (2006) reviewed assessment approaches and identified knowledge gaps in relation to risk assessment within Australia and, in particular, the Wet Tropics Bioregion. Webb noted that there had been no similar quantitative analyses as that applied by Kolar and Lodge (2002) using species' biological traits for tropical regions either in Australia or overseas. Bomford and Glover (2004), for Australia-wide introductions of non-native fishes, used a series of risk scores as variables based on climate match, overseas range size, number of global introductions and history of invasion success and a taxonomic risk score of a species based on invasion history of other species in the same genus and family. Using principal component analysis (PCA), they found that climate match and overseas range were the key predictors of establishment, while history of introduction success, taxonomic risk and overseas range were the key predictors using CART. Cross-validation, however, provided a much lower classification accuracy of 63.3% and 77.6% respectively, compared with a much higher values obtained by Kolar and Lodge (2002) for successful establishment of non-native fish in the Great Lakes, North America, of 87% (DA) and 94% (CART). In contrast, Kolar and Lodge (2002) used similar categorical variables, but also a large number of quantitative measures from life history attributes and environmental tolerances of each species.

To test the Kolar and Lodge assessment model for the Wet Tropics Bioregion Webb (2006) recommended the collection of basic ecological data from laboratory and field studies using standardised protocols to fill gaps in the literature on life history traits and physiological tolerances for all non-native fishes reported from fresh waters in tropical and sub-tropical Queensland.

The aims of the present study are:

- to apply the region-specific approach of Kolar and Lodge (2002) and develop a predictive model for non-native fish introductions in tropical northern Queensland fresh waters based primarily on life-history attributes and environmental tolerance data of previously introduced species;
- to identify key attributes of invasive species; and
- to test the classification accuracy of the model by comparison of analyses using data sets for introductions of non-native freshwater fishes in northern Queensland only and for the broader tropical/subtropical region of Queensland.

2.0. METHODS

This study applies the general quantitative model developed by Kolar and Lodge (2002) to identify key attributes of invasive non-native fish species reported in the Great Lakes region, North America. Kolar and Lodge (2002) defined three distinct invasion stages: establishment, spread and impact. At each stage, species were categorised (successful establishment/failed to establish; rapid dispersers/slow dispersers; nuisance/non-nuisance) and a suite of ecological characteristics compiled from various sources for each species. At each stage, statistical procedures were used to identify significant or key attributes of category membership for each species. In the present study, due to lack of historical information on natural dispersal of reported species in Queensland waters, analysis of the spread stage was not included in this study. The invasion stages (establishment and impact) defined by Kolar and Lodge (2002) were used in this study.

2.1. Identification and status of introduced, non-native fishes

The number of introductions of non-native fishes and their status in Queensland has been well documented (Arthington et al., 1999; Lintermans, 2004; Webb, 2008 in press). At least 15 out of 32 species have established populations in Queensland, while for northern Queensland waters only, at least 11 out of 22 species introduced have established. While only eight families are represented (Cichlidae, Poeciliidae, Osphronemidae, Cyprinidae, Cyprinodontidae, Percidae, Salmonidae, Cobitidae) among the reported introduced species, there are 26 genera, with a maximum representation of two species per genus. Of these reported species, five have been declared noxious in Queensland (Mozambique tilapia, spotted tilapia, mosquitofish, carp, oriental weatherloach). At least four other species (platy, swordtail, guppy and goldfish) have been reported as having adverse impacts within their introduced range (e.g., Englund, 1999; Lusk et al,. 2004; Chong and Whittington, 2005; Ruiz-Campos, 2007; Morgan and Beatty, 2007; Anon., 2008). At least five species (redhead cichlid, three-spot cichlid, blue-eye cichlid, redfin perch and tench) previously reported as introduced into Queensland waters (O'Connor, 1886, Lintermans, 2004; Webb, 2008 in press) were not included in model development due to a lack of sufficient ecological data in the literature or specimens available for tolerance trials (Table 1).

Table 1. Status of non-native freshwater fishes reported from northern Queensland (NQ) or Queensland (Q) waters and species used in physiological tolerance trials (critical minimum and maximum temperature (T), critical minimum respiratory oxygen threshold (O), salinity (S)).

Taxon	Species		Testing			Established	
			T	O	S	NQ	Q
Cichlidae	Mozambique tilapia	*Oreochromis mossambicus*	+			+	+
Cichlidae	spotted tilapia	*Tilapia mariae*	+	+	+	+	+
Cichlidae	Burton's haplochromis	*Haplochromis burtoni*	+	+	+	+	+
Cichlidae	Midas cichlid	*Amphilophus citrinellus*	+	+		+	+
Cichlidae	jewel cichlid	*Hemichromis lifalili*	+	+	+	+	+
Cichlidae	firemouth	*Thorichthys meeki*	+	+	+		
Cichlidae	convict cichlid	*Amatitlania nigrofasciatus*	+	+	+		
Cichlidae	pearl cichlid	*Geophagus brasiliensis*		+	+		
Cichlidae	blue acara	*Aequidens pulcher*	+	+	+		
Cichlidae	green terror	*Aequidens rivulatus*	+	+	+		
Cichlidae	green severum	*Heros severus*	+	+	+		
Cichlidae	oscar	*Astronotus ocellatus*	+		+	+	+
Cichlidae	redhead cichlid	*Vieja synspila*					
Cichlidae	three-spot cichlid	*Heros trimaculatus*					
Cichlidae	blue-eye cichlid	*Archocentrus spilurus*					
Osphronemidae	three-spot gourami	*Trichogaster trichopterus*	+		+	+	+
Cyprinodontidae	american flagfish	*Jordanella floridae*	+	+	+		
Poeciliidae	Platy	*Xiphophorus maculatus*	+	+	+	+	+
Poeciliidae	Swordtail	*Xiphophorus helleri*		+	+	+	+
Poeciliidae	Guppy	*Poecilia reticulata*	+			+	+
Poeciliidae	Mosquitofish	*Gambusia holbrooki*		+		+	+
Poeciliidae	sailfin molly	*Poecilia latipinna*	+				+
Salmonidae	brown trout	*Salmo trutta*					
Salmonidae	rainbow trout	*Oncorhynchus mykiss*					
Cyprinidae	Carp	*Cyprinus carpio*					+
Cyprinidae	Goldfish	*Carassius auratus*					+
Cyprinidae	rosy barb	*Puntius conchonius*	+	+	+		
Cyprinidae	tiger barb	*Puntius tetrazona*	+	+	+		
Cyprinidae	Tench	*Tinca tinca*					
Percidae	redfin perch	*Perca fluviatilis*					
Cyprinidae	white cloud mountain minnow	*Tanichthys albonubes*					
Cobitidae	oriental weather loach	*Misgurnus anguillicaudatus*					+

2.2. *Physiological tolerance trials*

Laboratory trials were conducted to collect physiological tolerance data (minimum and maximum temperature, maximum salinity and minimum hypoxia limits) for those species where comparable information was unavailable in the literature (see Table 1). For all tolerance trials, samples of 10 of each species to be tested were acclimatised to laboratory conditions in holding tanks for at least 10 days. Adults of small species and juveniles of medium to large species (maximum size <6cm) were used in the trials. Holding and experimental tanks were filtered and aerated and fish fed daily *ad libitum* on dried flakes and frozen bloodworm. Water quality in both sets of tanks was regularly monitored. Ammonia concentrations in thermal and salinity trials were minimised by use of ammonia neutralising agent and partial or complete water changes. Ethics approval was obtained for all experimental procedures from James Cook University Ethics Committee:

2.2.1. Thermal tolerances (maximum and minimum critical temperatures and temperature tolerance range)

Ten fish were placed individually in separate 10 l tanks established in two controlled temperature rooms. Room temperature was increased or decreased by 2°C every 76 hrs and the water temperature in each tank monitored. Water temperatures stabilised about 12 hours following each change. The response (feeding and swimming behaviour) of each fish at each change was also recorded until the critical (lethal) maximum or minimum temperature was reached. The difference between the maximum and minimum critical temperatures provided the maximum tolerance range of a species.

2.2.2. Salinity tolerance

Ten fish were placed individually in separate 10 l tanks in the laboratory at an ambient temperature of 28°C. Salinity in each tank was changed every 76hrs by 4ppt using synthetic sea salt (Ocean Nature by Aquasonic). Salt was dissolved in water removed from the experimental tank and slowly reintroduced close to the aerator with further *in situ* mixing using a spatula until the target concentration was reached. The response of each fish (feeding and swimming behaviour) at each change was also recorded until the critical (lethal) salinity or a final 35ppt salinity concentration was reached. The mean critical salinity was expressed as a proportion of 35ppt and therefore provided a measure of a species' tolerance within a salinity range from freshwater to full strength seawater.

2.2.3. Dissolved oxygen concentration (critical minimum respiratory oxygen threshold)

Trials were conducted in the laboratory using the method described by Butler et al. (2007). Ten individuals of each test species were acclimated to normoxia, water quality and temperature conditions (28°C) prior to the tests. Fish were each placed in a small perspex cell within a larger aquarium tank and positioned within the field of focus of video cameras and allowed to adjust to confinement before the test commenced. The large aquarium was then sealed and fish allowed to deplete oxygen levels in the tank, with additional drawdown by circulating water through a separate tank containing fish

(Mozambique tilapia), the numbers of which could be changed to control the duration of the test to approximately three hours. Dissolved oxygen concentrations and pH were monitored throughout the test and the latter adjusted to maintain levels above 6.5. Tests were terminated when observed gill ventilation rate (GVR) of the most tolerant individual began to decline which is indicative of respiratory stress.

Precise counts of GVR were obtained from playback of high resolution digital video footage. Plots of GVR (obtained from beat rate and amplitude) versus DO concentration were prepared for each individual test subject and the peak GVR was estimated by interpolation. The acute trigger value (ATV) was then taken as an average of GVR values for the ten test subjects and represents the critical minimum respiratory oxygen threshold. According to Butler et al. (2007), this method provides a basis for comparing the physiological requirements of fish. The ATV represents the point which will eventually be lethal and at which fish will be forced to employ a variety of high risk and potentially unsustainable survival strategies (e.g., avoidance, aquatic surface respiration (ASR), where fish irrigate the gills with water in the boundary layer with the atmosphere that has a higher concentration of dissolved oxygen, or air gulping by species such as anabantids and lungfish with specialised auxiliary respiratory stuctures).

Butler et al (2007), noted that there are many factors in the field which determine hypoxia in the wild besides physiological attributes of the species, including fish size and ability to access oxygen in the boundary layer (by ASR). They noted that large fish tend to be more hypoxia tolerant than small fish, while the latter are more able to perform ASR and survive conditions normally below their physiological limits. Odum and Caldwell (1955), for example, found the poeciliid, *Gambusia affinis,* survived normally lethal hypoxic conditions in an anaerobic spring (2.9% oxygen saturation at 22.5°C) as long as fish had access to the surface boundary layer. Allowance for size factors were made by standardising the size of test subjects and also by including a measure of maximum size (standard length) as a weighting variable in the univariate and multivariate statistical analyses. Where species were not available for testing, DO tolerance values were obtained from the literature which were, as far as possible, equivalent to the test values.

2.2.2.4. Other species attributes

Information required for model development identified by Webb (2006) on life history characteristics, habitat needs, and aspects of invasion history and human use of each species were obtained from the literature (peer-reviewed journals, reports, electronic databases and websites and reference texts) and, with the physiological tolerance data from the laboratory trials. These data then provided sixteen input variables to test the model (Table 2).

The absence of detailed cladistic information for all species precluded sophisticated analyses to assess taxonomic bias due to phylogenetic similarity. However, a weighting variable was included to reduce this likelihood by ranking fish families according to degree of derived characters, from the most ancient to the most derived based on the classification of Nelson (2006)(xvi-xvii). Where several values were obtained from the literature for a given attribute, an average of these values was used in model development. All information sources accessed are listed in the Reference Section II of this report.

2.3. Data analysis

Following the procedure of Kolar and Lodge (2002), for the Establishment Stage, fish were divided into two groups: those which had successfully established self-maintaining populations and those which had failed to establish. Insufficient data were available to define a Spread Stage for all species and was not included in the analysis. For the Impact Stage, all established species, based upon information available in the literature on their impacts in their introduced ranges, were classified as either ‘Nuisance’ or ‘Non-nuisance’ species. The established species in Queensland and northern Queensland are listed in Table 1 (Methods section).

Exploratory one-way ANOVAs relating success or failure to establishment of non-native fishes in northern Queensland and for the larger Queensland region were conducted for each variable. Significant variables only for each analysis were identified and tabled. Two multivariate procedures: discriminant analysis (DA)(SPSS) and categorical and regression tree analysis (CART)(Salford Systems Pro ExV6.0) were applied to northern Queensland and Queensland data sets. DA was used for the establishment and impact stages, while CART was used only for the establishment stage due to small sample sizes. Both DA and CART procedures provided cross-validation of classification results to determine the degree of model stability.

The goal of these analyses was to identify suites of variables that best discriminate ‘successful’ from ‘failed’ fishes at the establishment stage and ‘nuisance’ from ‘non-nuisance’ species at the impact stage. Using this suite of variables, the data were re-analysed to compare classification accuracy of the different procedures (ANOVA, DA and CART) for each stage and for different data sets (northern Queensland and Queensland) using all variables (Q1 and NQ1 – see Table 2) or limited to use of continuous variables derived directly from measurement of species physical or physiological characteristics (Q2 and NQ2 – see Table 2, Variable Reference No. 2).

DA and CART analyses provide a percentage of species correctly or incorrectly classified to their designated category for each stage (i.e., ‘Established’ or ‘Not-established’, ‘Nuisance’ or ‘Non-nuisance’) for model development, and also with cross-validation for model testing. The relative importance of variables in DA is provided by the function coefficients and in CART by a 100 point scale from 0 (least important) to 100 (most important).

Table 2. Attribute variables for for non-native freshwater fish species used in model development

Species characteristic (units)	Type of variable (Reference No.)		Species characteristic evaluation
Maximum temperature tolerance (°C)	continuous	2	Maximum lethal temperature limit
Minimum temperature tolerance (°C)	continuous	2	Minimum lethal temperature limit
Temperature tolerance range (°C)	continuous	2	Maximum range of temperature inhabited (T_{max} – T_{min})
Salinity tolerance (ppt)	proportion	2	Lethal salinity tolerance measured as a proportion of full strength seawater (35ppt)
Minimum respiratory oxygen threshold (% oxygen saturation)	continuous	2	Minimum critical respiratory tolerance threshold represents the environmental oxygen saturation level at which a species maximum ventilation rate occurs at a specified temperature (28°C) (for tolerance trials); or comparable value obtained from literature
Egg diameter (mm)	continuous	2	Average diameter of mature ova
Parental care	Categorical		Ranked according to degree of parental care (1 = broadcast spawner; 2 = nest/egg guarder; 3 = livebearer/mouthbrooder)
Resilience	Categorical		Population doubling time ranked: 1=>4.4yrs; 2 = 1.25-4.4yrs; 3 = <1.25yrs). Data obtained from Froese and Pauly (eds)(2008)
Vulnerability	Categorical		A measure of a species intrinsic ability to respond to population pressure associated with biotic and abiotic variability in the environment devised by Cheung et al. (2005) based on life history parameters. Values on a scale of 0-100 (low to high response capacity) for each species obtained from Froese and Pauly (eds)(2008)
Size (cm)	continuous	2	Average standard length at maturity (SL)
Diet	Categorical		A matrix was constructed to combine diet breadth and diversity using habitat from which food was derived (terrestrial, water column, benthic) and food size and type(detritus, micro- or macro-plants, micro- or macro-invertebrates, vertebrates). The number of squares from which each species consumed food items, based on literature data provided a measure of diet breadth and diversity.
Size of native range	continuous	2	Number of degrees of latitude of native range (data obtained from Froese and Pauly (eds)(2008)
Climate match: absolute range overlap	Proportion		Proportion of overlap between absolute native latitudinal range with introduced latitudinal range (NQld: 11°S - 20°[9°]) or Qld: 11°S-29°S[18°]) for a species
Human use	Discrete		Sum of human uses of a species from: recreational/sports fishery; commercial fishery; forage species; aquaculture; aquarium/ornamental; biocontrol; baitfish; research
Propagule pressure I: History of introduction	Discrete		Total number of international introductions of a species
Propagule pressure II: History of establishment	Proportion		The proportion of successful international introductions of a species
Taxon	categorical		Families of fish species ranked according to degree of derived characters

3.0. RESULTS

ANOVAs for both northern Queensland and Queensland data sets provided a similar suite of variables that identified successful species in the establishment stage. Successful species had lower critical minimum respiratory (oxygen) thresholds, had greater levels of parental care, and had a history of more successful international introductions than species that failed to establish. For the impact stage, for Queensland data only, 'nuisance' species had a greater temperature tolerance range, lower critical minimum temperature tolerance, and greater native latitudinal range and a history of more international introductions than 'non-nuisance' species (Table 3).

Table 3. ANOVA summary of significant variables for establishment and impact of non-native fishes in northern Queensland and Queensland fresh waters

	ESTABLISHMENT			
	variable	F	d.f.	p
NQ1	critical minimum oxygen threshold (% O sat.)	9.548	1,19	0.006
	parental care	8.117	1,19	0.010
	% successful international introductions	28.27	1,19	<0.001
NQ2	critical minimum oxygen threshold (% O Sat.)	9.548	1,19	0.006
Q1	critical minimum oxygen threshold (% O Sat.)	14.249	1,23	0.001
	parental care	4.932	1,23	0.036
	% successful international introductions	31.846	1,23	<0.001
Q2	critical minimum oxygen threshold (% O sat.)	14.249	1,23	0.001
	IMPACT			
NQ1	Insufficient data for analysis	-	-	-
NQ2	Insufficient data for analysis	-	-	-
Q1	native latitudinal range (° lat.)	4.775	1,12	0.049
	total number of international introductions	6.615	1,12	0.024
	critical minimum temperature (° C)	5.621	1,12	0.035
	temperature tolerance range (° C)	4.649	1,12	0.050
Q2	critical minimum temperature (° C)	5.621	1,12	0.035
	temperature tolerance range (° C)		1,12	0.050

Discriminant Analyses using both continuous and categorical variables provided the highest level of classification accuracy for the establishment stage in northern Queensland and Queensland (81% and 92% with cross-validation respectively) compared with the use of directly-measured, continuous variables only (66.7% and 72% respectively). In both cases, the higher level of classification accuracy was obtained with the larger sample size (Queensland data set: n = 27). However, a moderately high level of classification accuracy (72% with cross validation) was still obtained from analysis of

Table 4. Classification summary for DA and CART analyses of establishment success

						Wilks lambda		Correct classify %
		Stage	Wilks lambda	Chi Sq.	d.f.	Sig.	Model develop	Cross validation
DA	NQ1	establish	0.026	41.778	15	<0.001	100	81
DA	NQ2	establish	0.456	12.174	7	0.095	85.7	66.7
DA	Q1	establish	0.068	40.339	16	0.001	100	92
DA	Q2	establish	0.371	18.828	8	0.016	84	72
DA	NQ1	impact	0.011	22.476	10	0.013	100	50
DA	NQ2	impact	0.366	6.534	7	0.479	91.7	41.7
DA	Q1	impact	0.180	10.294	12	0.590	100	64.3
DA	Q2	impact	0.409	7.147	8	0.521	85.7	57.1
CART	NQ1	establish					90.5	76.2
CART	NQ2	establish					85.7	85.7
CART	Q1	establish					92.6	85.2
CART	Q2	establish					88.9	88.9
CART	NQ1	impact					91.7	75.0
CART	NQ2	impact					83.3	58.3
CART	Q1	impact					86.7	66.7
CART	Q2	impact					86.7	73.3

the larger Queensland data set using only the directly-measured continuous variables. Three of the four analyses of impact stage data sets provided non-significant results that reflected the low sample sizes (northern Queensland: n = 12; Queensland n = 15) and the low level of classification accuracy (41.7 and 64.3% respectively).

For CART analyses, the highest classification accuracy was obtained for the establishment stage (76.2 - 88.9%) compared with the impact stage (58.3 – 75.0%) irrespective of sample location and reflects the smaller sample size of the latter. For both sets of analyses, the highest values were obtained for the larger Queensland data sets and use of continuous, directly-measured variables only (establishment stage: 88.9%; impact stage: 73.3%).

From all analyses that provided a significant class differentiation, ten key predictors were identified for establishment success. Of these, seven were continuous variables which were measures of environmental tolerance (temperature, dissolved oxygen concentration), habitat need (overseas range) and physical characteristics (body size, egg diameter) and life history attributes (extinction vulnerability), while three variables were measures of human use (previous invasion history and use) (Table 5).

Table 5. DA function coefficients for key variables at establishment and impact stages for non-native fishes in northern Queensland and Queensland fresh waters.

ESTABLISHMENT STAGE	DATA SET		
	NQ1	Q1	Q2
critical maximum temperature (°C)	2.057	-6.926	-5.651
critical minimum temperature (°C)		9.062	6.608
temperature tolerance range (°C)		9.511	7.287
critical minimum oxygen threshold (% O sat.)		-1.907	-1.619
egg diameter (mm)	2.700		-1.599
vulnerability	-4.628		
human use		1.947	
total number of global introductions		-1.959	
percent successful global introductions	-4.029		
size	5.831		
IMPACT STAGE			
Critical minimum temperature (°C)	8.970		
Critical minimum oxygen threshold (% O sat.)	6.892		
Native latitudinal range (° latitude)	11.223		
Range overlap	-12.039		
Egg diameter	-7.809		
size	-7.465		

For the impact stage, all of the six important predictors for identifying nuisance species for northern Queensland were continuous variables. Four were measures of environmental tolerance and physical attributes (minimum temperature, hypoxia threshold, egg size and body size) and two were measures of habitat need (native range size and absolute range overlap between native and introduced range).

For the overall CART analyses, nine key predictors were identified for successful establishment. Six were continuous, directly-measured variables: four environmental tolerances (three temperature and dissolved oxygen concentration), two physical attributes (body size and egg diameter), with one categorical variable (parental care) and two measures of human use or propagule pressure (number and success of previous introductions). Successful species had lower critical minimum respiratory oxygen thresholds, lower minimum and higher maximum temperature tolerances and larger eggs than unsuccessful species. Successful species also invested in more parental care and had more introductions and a higher percent of previous successful introductions than unsuccessful species. For the impact stage, seven key predictors were identified, six of these were continuous, directly measurable variables: four environmental tolerances (maximum, minimum temperature and temperature tolerance range and low dissolved oxygen concentration), one physical attribute (body size), one measure of habitat need (native range size) and one measure of previous invasion history (or propagule pressure) (total number of previous international introductions)(Table 6).

Table 6. Summary of important variables from CART analysis to predict establissment success and impact of non-native freshwater fishes in northern Queensland or Queensland.

STAGE	DATA SET			
ESTABLISHMENT	Relative importance			
	NQ1	NQ2	Q1	Q2
Critical maximum temperature (°C)		25.4		17.58
Critical minimum temperature (°C)		34.1		
Temperature tolerance range (°C)				
Critical minimum respiratory threshold (% O sat.)	39.0	100.0	50.0	100.0
Egg diameter (mm)		25.4		15.9
Body size (SL)(cm)	20.0			
Parental care	34.4		24.1	
Total number of previous international introductions	28.0		42.4	
% successful previous introductions	100.0		100.0	
IMPACT	NQ1	NQ2	Q1	Q2
Critical maximum temperature	46.7	66.7	78.6	78.6
Critical minimum temperature	36.0	51.4	58.9	58.9
Temperature tolerance range	70.0	100.0	100.0	100.0
Critical minimum respiratory threshold (% O sat.)		18.2	44.9	
Body size (SL)(cm)	46.7	18.2		19.6
Size of native (latitudinal) range	36.0			
Total number of previous international introductions	100.0		100.0	

Decision trees were constructed using the important variables identified in each analysis. For the establishment stage the suite of predictor variables were the same for northern Queensland or Queensland data sets when directly-measured, continuous variables only were used, and the same when directly-measured, continuous and categorical variables were used. For the analysis using directly-measured, continuous variables, the strongest predictors were critical minimum respiratory threshold and critical minimum and maximum temperature, while for the analysis using both types of variables, the strongest predictors were history of successful introductions elsewhere and human use, although this decision tree also included directly-measured continuous variables (critical maximum temperature or critical minimum respiratory threshold and egg diameter) as strong predictors (Figure 1 and 2).

For the impact stage, using directly-measured continuous variables only, temperature tolerance range, salinity tolerance, critical minimum respiratory threshold and egg diameter provided optimal classification (Figure 3), while temperature tolerance range, number of previous international introductions and human uses, and egg diameter provided optimal classification when both types of variables were used (Figure 4).

Figure 1. Decision tree (CART) for establishment success of non-native fishes in northern Queensland or Queensland fresh waters (directly-measured, continuous variables only).

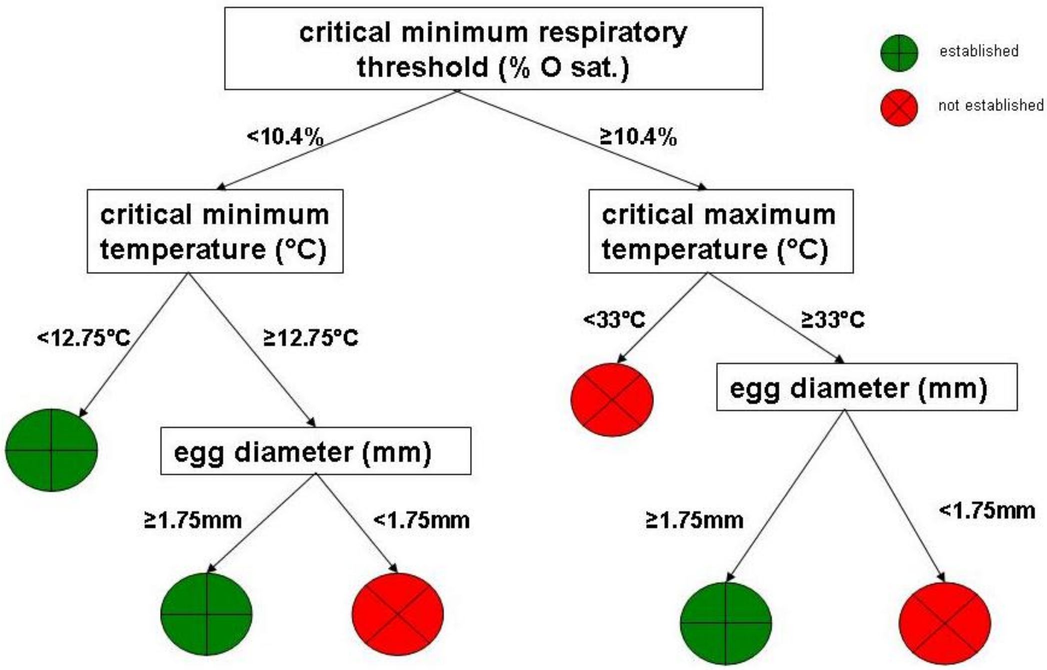


Figure 2. Decision tree (CART) for establishment stage of non-native fishes in northern Queensland or Queensland fresh waters (directly-measured, continuous and categorical variables).

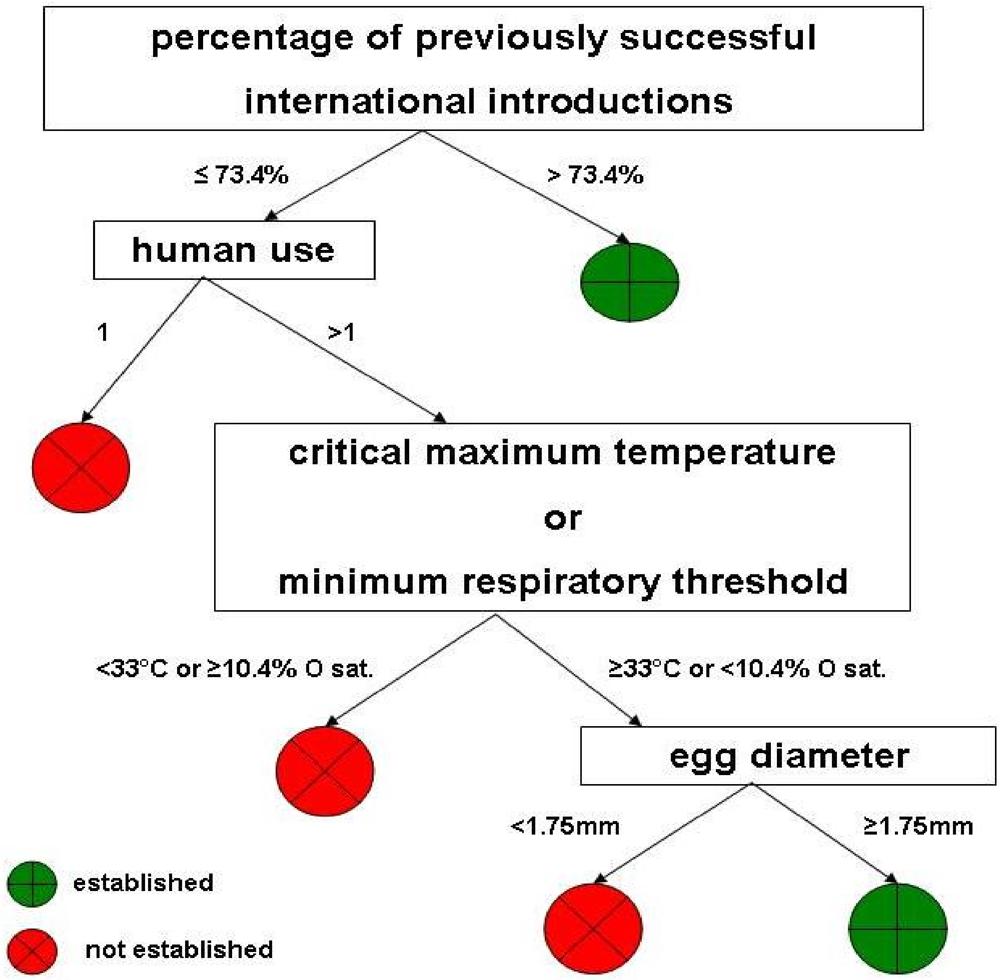


Figure 3. Decision tree (CART) for impact stage of non-native freshwater fishes in northern Queensland or Queensland (directly-measured, continuous variables only).

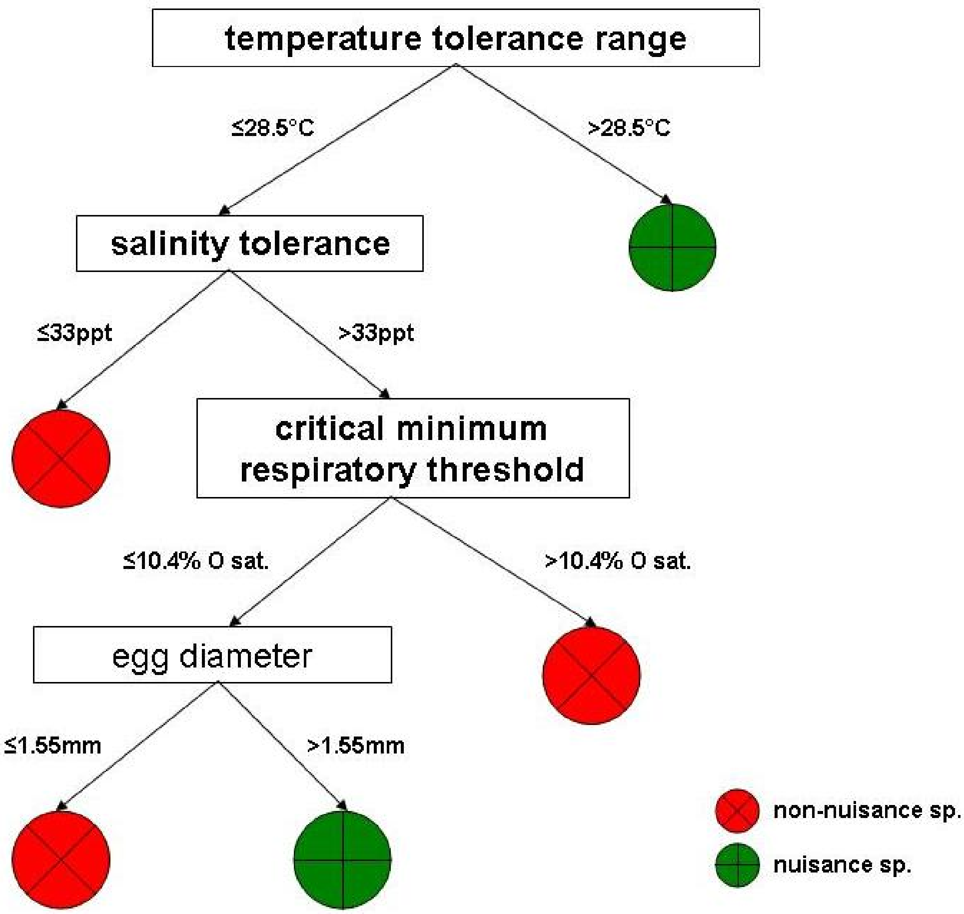
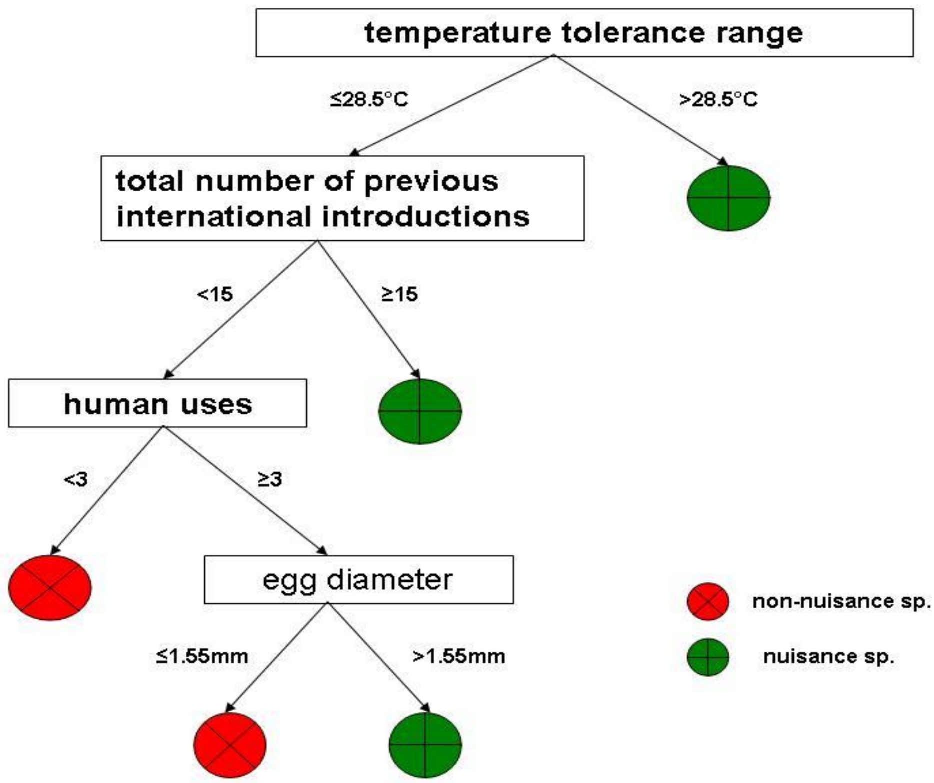


Figure 4. Decision tree (CART) for impact stage of non-native freshwater fishes in northern Queensland or Queensland (directly-measured, continuous variables and categorical variables combined).



4.0. DISCUSSION

The model identified a suite of attributes characteristic of non-native freshwater fish species with a high risk of establishment either within tropical northern Queensland or the broader tropical-subtropical climate region of Queensland. The larger Queensland sample size improved the model's predictive power and, since many of the established species occur throughout the state, the model provides a single risk assessment procedure for Queensland fresh waters. The analyses also provided a high level of classification accuracy (CART: 88.9%; DA: 92% with cross-validation) for establishment success, similar to that obtained by Kolar and Lodge (2002) and higher than the obtained by Bomford and Glover (2004) using a similar approach, but with only categorical variables (CART: 77.6% and Principal Component Analysis (PC): 63.3% with cross validation). The model used by Kolar and Lodge (2002) and also in this study, incorporated a weighting variable for taxonomic similarity, while no similar variable was used by Bomford and Glover (2004). These authors used a continental rather than regional scale analysis, as in the present study, and only assessed on the basis of a literature review, rather than testing possible factors that are predictive of establishment success. While recognising that four introduced species, European carp, goldfish, gambusia and weatherloach have wide temperature tolerances and are resistant to hypoxia, Bomford and Glover (2004) stated that there was only anecdotal evidence to support any link between these physiological attributes and establishment success. The present study demonstrated that these attributes are key factors in establishment success and as predictors of 'nuisance' species in tropical and sub-tropical fresh waters of Queensland.

Based on their literature review, Bomford and Glover (2004) also devised a series of indices to represent five key factors for establishment success (number of international release events and successful introductions elsewhere, climate match between a species native and introduced range; overseas geographic range size and taxonomic similarity), none of which are directly measurable attributes of a species. The assessment has limited regional value as it is at a continental scale. Five species reported as extreme to high establishment risk (redfin perch, tench, rainbow trout, brown trout, convict cichlid) have failed to establish populations in Queensland, the latter three species also failing to establish in northern Queensland. Only the sailfin molly has established populations (in south-eastern Queensland), although it has failed, after one introduction, to establish in northern Queensland. One species, the blue acara, classed as a moderate risk has failed to establish after several introductions while Burton's haplochromis, classed as a low risk but with no previous invasion history, has established a population in the Ross River catchment and is starting to disperse locally. The probability of establishment may be influenced by a range of factors, such as local site conditions, stochastic processes (such as timing of introduction) as well as low 'propagule pressure', i.e., numbers of introduction sites and or number of individuals released (Williamson, 1996; Claudi and Leach, 2000; Fuller and Drake, 2000). Webb (2003) reported a strong correlation between the percentage of established non-native fish species with the known frequency of their introductions in northern Queensland. In most instances such site- or region-specific information is not available or is difficult to estimate, with reliance on more indirect indices – such as number and success of international introductions which represents a limiting factor on predictive power of such models.

There is currently limited opportunity, due to lack of ecological data, to test the model on 'potential' invaders in northern Queensland or Queensland waters. However, two of the 'failed' non-native fish species mentioned above, the redfin perch and tench, were not included in the model development due to lack of data for all the variables used, but had comparable values from the literature for the environmental tolerance and life history variables identified in the analyses as strong predictors. There have been several unsuccessful attempts to introduce redfin perch into Queensland (O'Connor 1886; McCulloch 1929; Weatherley 1963) and one unsuccessful attempt to introduce tench (O'Connor 1886), although in the latter case, this was most probably due to only three individuals being released into Gold Reservoir, near Brisbane. Interestingly, using both of the CART decision trees (Figures 1 and 2), the redfin perch would be a low risk (contradicting Bomford and Glover's assessment) while tench would be a high risk of establishment (in agreement with Bomford and Glover's assessment). For the impact stage, using both decision trees (Figures 3 and 4), tench would be a low risk of becoming a nuisance species.

Both DA and CART demonstrated that continuous variables from direct measurement of species attributes, particularly those that are more easily obtainable (e.g., physiological tolerances, size, egg diameter) compared with other life history characteristics (e.g., diet, growth, longevity, fecundity) can be used to successfully identify species with a high risk of establishment. This is particularly important where little or no prior information is available for a species including life history parameters, native range (as an indirect measure of climate/habitat requirement or matching) and invasion history (as an index of propagule pressure).

Successful application of the risk assessment model requires information obtained from standardised methodologies and units of measurement. The information can be obtained from a certified facility (e.g., university or government agency) and, in the case of assessment for a proposed new import, the costs should be borne by the proponent. It can be argued that costs associated with developing and applying a more consistent and powerful screening tool are far outweighed by those associated with subsequent management of feral populations of a species otherwise mis-identified as a 'low risk'(Pimentel, 2002).

The small sample size of established species (12 for northern Queensland and 15 for Queensland) and limited information on natural spread and potential impacts of some species used in the analysis affected the predictive power of the model for identifying rapid dispersers and classification accuracy for discriminating 'nuisance' and 'non-nuisance' species. The rate of spread after initial introduction of non-native fishes throughout Queensland has been due largely to human translocation with lack of catchment interconnectivity acting as a barrier to large scale natural dispersal – compounded by a lack of historical data for all species. Some localised dispersal has occurred particularly during periods of flooding or creek hopping via coastal waters by more euryhaline species although, again, range increases may be due to human translocation rather than natural dispersal.

Introduced species impacts, including those of fish, have largely been defined in economic rather than ecological terms, and in the latter case, such impacts may be part of a complex interaction of factors that are manifest in the long term and are difficult to quantify. They may also be part of a broader environmental dynamic, where introduced species are replacing rather than displacing native fishes due to changes, such as habitat degradation, where the altered conditions favour the invaders at the expense of native species. In many cases, information on impacts of species is lacking. This could have affected the outcomes of model development in regard to species class assignment, i.e., to either the ‘nuisance’ or ‘non-nuisance species’ category. Two recent introductions in northern Queensland, Burton’s haplochromis and the three-spot gourami, have little or no information available on their impacts. In the case of Burton’s haplochromis, the species has no previous invasion history, but has wide ecological tolerances, is aggressive and carnivorous, while the three-spot gourami, one of the most rapidly spreading species in northern Queensland, is also hardy and is carnivorous and aggressive, but little is known of its possible impacts on other species within its introduced range elsewhere. Nevertheless, the analysis showed that extreme eurythermal, euryhaline and euryoxic attributes are strong predictors of nuisance species whether in fresh waters of northern Queensland or the broader tropical/sub-tropical region of Queensland. Such attributes can be directly measured, are quantifiable and therefore subject to independent statistical analysis.

The model identified species’ attributes that are strong predictors of establishment success and impact, and improved classification accuracy by inclusion of directly measured ecological variables, especially for physiological tolerances, and provides a more reliable assessment of species compared with deductive models where there is no previous invasion history. While the objective in risk assessment is to discriminate the successful from the failed invader, and identify the likely nuisance species, ecological systems are dynamic and not entirely predictable. Given the uncertainty inherent in assessment processes and lack of ecological information on potential invaders, application of such models, in the absence of further refinement, should be guided by the precautionary principle to avoid false positives – i.e., mis-classifying high risk species. While the successful application of this model is based on gathering quantitative ecological information, the associated costs are far outweighed by the costs associated with managing the impacts of some of these not-so-welcome ‘guests’.

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6.0 APPENDIX A

Table A Ecological tolerance data obtained from laboratory trials for non-native fishes reported from Queensland fresh waters (lit: data available in literature)

Species	Max temp (°C) max(mean)	Min temp (°C) min(mean)	Salinity (ppt) max(mean)	Oxygen (% sat.) min(mean)
Oreochromis mossambicus	lit	8.9(9.2)	lit	lit
Tilapia mariae	lit	lit	lit	7.3(10.1)
Haplochromis burtoni	38.9(38.4)	10.5(10.8)	32.0(28.0)	7.8(8.6)
Amphilophus citrinellus	39.1(38.8)	11.1(12.7)	lit	9.4(9.5)
Hemichromis lifalili	38.9(38.3)	9.2(10.2)	35.0+(35.0)	4.0(6.1)
Thorichthys meeki	40(39.7)	10.5(10.7)	28.0(27.2)	9.4(13.4)
Amatitlania nigrofasciatus	38.3(37.8)	9.9(10.5)	35.0(35.0)	9.8(13.2)
Geophagus brasiliensis	lit	lit	35.0+(35.0)	7.0(10.6)
Aequidens pulcher	40.6(40.4)	12.5(13.3)	35.0(31.5)	6.2(7.4)
Aequidens rivulatus	37.8(37.4)	11.2(11.7)	28.0(28.0)	5.6(7.1)
Heros severus	40.2(39.7)	12.6(12.8)	24.0(22.8)	9.9(11.2)
Astronotus ocellatus	40.1(38.7)	12,7(14.1)	24.0(23.2)	lit
Puntius conchonius	lit	lit	20.0(20.0)	6.6(10.8)
Puntius tetrazona	36.9(36.1)	12.8(13.1)	18.0(17.2)	10.1(12.6)
Jordanella floridae	40.4(40.2)	6.6(7.2)	lit	9.9(11.0)
Xiphophorus maculatus	38.1(37.6)	7.6(8.4)	35.0+(30.2)	9.0(11.0)
Xiphophorus helleri	lit	lit	35.0+(26.8)	6.4(10.1)
Poecilia reticulata	lit	9.9(10.5)	lit	lit
Poecilia latipinna	38.7(36.3)	8.7(12.6)	lit	lit
Trichogaster trichopterus	38.3(37.7)	10.2(10.5)	35.0+(34.4)	lit