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Integration of Functional Systems:
Assessing the Use of the Locomotor System During Prey Capture in Fishes

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Evolution, Ecology, and Organismal Biology

by

Emily Alison Kane

August 2014

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The Dissertation of Emily Alison Kane is approved:

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DEDICATION

This dissertation is dedicated to my husband, Josh.
You are so patient and supportive.
I love you.

ABSTRACT OF THE DISSERTATION

Integration of Functional Systems:
Assessing the Use of the Locomotor System During Prey Capture in Fishes

by

Emily Alison Kane

Doctor of Philosophy, Graduate program in Evolution, Ecology, and Organismal Biology
University of California, Riverside, August 2014
Dr. Timothy E. Higham, Chairperson

Organismal complexity is often reduced to individual systems, but organisms function as an integrated whole and reductionist studies cannot address the constraints imposed by systems working together to perform a function. In this dissertation, I use integration between locomotor and feeding performance during prey capture in fishes as a model system for understanding complex behaviors and their ecological relevance.

First, I demonstrate the empirical utility of integration for describing emergent differences between species. I utilize two species of Pacific marine sculpins capturing live amphipod prey, and confirmed that species were similar in feeding behaviors but different in their use of locomotion during prey capture. This resulted in differences in integration that reflect ecological divergence that would not be apparent in feeding behaviors alone.

Second, I demonstrate that differential capture success is due to differences in predator accuracy. I utilize centrarchid sunfishes to develop a non-invasive model of suction volume and accuracy and apply this model to 3D feeding kinematics of three

predators capturing two prey types. Not only did accuracy vary across species, but so did the ability to modulate the shape of the ingested volume of water, leading to a direct effect on predator capture success.

Finally, I expand the techniques for quantifying behavioral integration to multivariate space and assess the causes and consequences of integration using a single centrarchid predator capturing two prey types. Partial least squares correlations describe multivariate integration and demonstrate that predators rely on performance variables differentially for each prey type. These differences are then reflected in patterns of integration and predator accuracy across prey types.

This dissertation advances our understanding of how organismal integration and complexity act to drive performance and ecology. I demonstrate that performance integration is real and can be quantified and establish the empirical and ecological relevance of performance integration. Integration and organismal complexity may be one of the next scientific frontiers, and this dissertation provides a first step in exploring this new direction.

TABLE OF CONTENTS

1. Introduction	1
References	5
2. The Utility of Integration in Feeding Biomechanics	
Introduction	9
Methods	13
Results	19
Discussion	28
References	36
3. The Influence of Approach Accuracy on Predator Success	
Introduction	41
Methods	48
Results	55
Discussion	66
References	75
Supplemental methods	85
4. The Causes and Consequences of Integration	
Introduction	90
Methods	96

Results	105
Discussion	118
References	130
5. Conclusions	140
References	143

LIST OF TABLES

Table 2.1	21
Mean kinematic variables for sculpins	
Table 2.2	27
Variable correlations with principal component axes	
Table 3.1	51
Species, sample sizes, and capture success rates for 3D kinematic dataset	
Table 3.2	56
Student's t-test for reference and modeled IVW parameters	
Table 3.3	56
Model selection statistics for predicting IVW dimensions	
Table 3.4	57
Comparison of predicted length with and without gape included	
Table 3.5	60
Mean 3D kinematics and modeled IVW parameters	
Table 4.1	106
Mean kinematics for each prey type	
Table 4.2	109
Variable loadings	
Table 4.3	116
Univariate correlations between locomotor and feeding variables	

LIST OF FIGURES

Figure 2.1	16
Digitized anatomical landmarks and kinematic measurements for sculpins	
Figure 2.2	20
Relationship between mouth area and total length	
Figure 2.3	22
Mean velocity and acceleration profiles	
Figure 2.4	24
Integration (correlation) of locomotor and feeding variables	
Figure 2.5	26
Principal component analysis of locomotor and feeding kinematics	
Figure 3.1	43
Schematic of calculation of ingested volume of water	
Figure 3.2	45
Comparison of reference and modeled IVW shapes	
Figure 3.3	52
Digitized anatomical landmarks for centrarchids	
Figure 3.4	58
Relationship between predicted and actual values of IVW length and height	
Figure 3.5	61
3D reconstruction of the ingested volume of water	

Figure 3.6	62
Species means for H:L ratio and accuracy index	
Figure 3.7	63
Position of the prey relative to the modeled IVW in frontal view	
Figure 3.8	65
Logistic regression of the probability of capture success	
Figure 4.1	94
Comparison between multivariate correlation techniques	
Figure 4.2	97
Digitized anatomical landmarks for bluegill	
Figure 4.3	99
Representative non-evasive prey kinematics	
Figure 4.4	100
Representative evasive prey kinematics	
Figure 4.5	108
Partial least squares correlations and loadings	
Figure 4.6	113
Principal components analysis correlations and loadings	
Figure 4.7	117
Relationship between integration and accuracy	

CHAPTER 1:

Introduction

Studies of organismal diversity are common in comparative biomechanics, where questions are often centered on the morphology-performance-fitness paradigm popularized by Arnold (Arnold, 1983). Performance refers to the ability to perform an ecologically relevant task (Irschick et al., 2008), and can be quantified using kinematics such as locomotor acceleration during an escape behavior (Law and Blake, 1996; Walker et al., 2005; Herrel and Bonneaud, 2012) or mechanics such as bite force during prey capture behaviors (McBrayer, 2004; Herrel et al., 2005; Huber et al., 2005; Marshall et al., 2012). Although some researchers have examined direct links between performance and fitness (e.g. Jayne and Bennett, 1990; Walker et al., 2005; Husak, 2006; Langerhans, 2009), measuring fitness of biomechanical traits in the field is difficult, and other researchers have focused on the relationship between morphology and performance instead (Webb, 1978; Losos and Sinervo, 1989; Walker and Westneat, 2002; Blake, 2004; Carroll et al., 2004; Wainwright et al., 2007; Domenici et al., 2008). Because of its central role in explaining organismal form and the expectation of evolutionary consequences, performance has played a central role in comparative biomechanics, where differences at this level are used to characterize differences in both morphology and fitness.

Although performance has been useful for describing differences between and among species, studies tend to reduce organismal complexity to individual functional systems,

such as locomotion or feeding. However, a more holistic idea is that organisms result from integrated levels of complexity (Bayliss, 1921; Olson and Miller, 1958; Gould and Lewontin, 1979; Zweers, 1979; Seaborg, 1999; Pigliucci, 2003), and systems can work together to perform a common ecologically relevant task. Because parts do not often function independently, significant aspects of evolutionary change can be overlooked when these systems are considered separately (Olson and Miller, 1958; Zweers, 1979). In fact, the correlated function of parts may be more beneficial to understanding organismal evolution than understanding the function of the individual parts (Bayliss, 1921; Gould and Lewontin, 1979). In a recent review of selection on performance traits, Irschick and others highlight that future work should assess selection on multiple traits, given that performance does not evolve independently from other features of the organism (Irschick et al., 2008). However, performance traits across systems are not likely additive, but rather comprise a tradeoff that must be mitigated during integrated behaviors (Ghalambor et al., 2003; Ghalambor et al., 2004; Walker, 2007; Irschick et al., 2008; Walker, 2010). In this case, neither measure of system-level performance adequately describes the emergent level of performance when both systems act together. Therefore, integration across system-level performance measures could be used as a more holistic and evolutionarily relevant measure that would encompass the interrelationships present across systems. However, quantifying performance in a system during dynamic behaviors is difficult and most researchers simplify these behaviors by constraining the organism. For this reason, combining two performance measures across integrated behaviors has been understudied.

This dissertation will examine the role of performance integration in empirical and ecological contexts to determine the importance of integration for organism function and survival. Performance integration is defined as the pattern of covariance between performance traits of two or more functional systems, and is an emergent property of the two systems operating together. Fishes will be used as a model system for understanding performance integration, as the locomotor system has a strong role in prey capture (Higham et al., 2005; Higham et al., 2006; Higham, 2007b; Higham et al., 2007). Suction is only useful for short distances (Svanbäck et al., 2002; Day et al., 2005), so locomotion is expected during the approach when fishes swim toward their prey (Wainwright et al., 2001). Locomotion may also be important for rapid deceleration and braking during or after prey capture (Higham, 2007b; Higham, 2007a). Webb (1984) noted that locomotor behavior can influence prey capture success in fishes, and recently, aspects of the locomotor system have been linked to performance of the feeding system (Higham, 2007b). Coordination with the feeding system would be relevant for predators because this integration would ensure the proper timing and positioning (accuracy) of the predator relative to the prey (Drost, 1987; Higham et al., 2006; Nauwelaerts et al., 2008) so that prey would be encountered within a given distance from the mouth at the time when suction is strongest and gape is the proper size to fit the prey. Therefore, integration likely has a role in predator accuracy, which in turn directly relates to prey capture success (Drost, 1987). In this way, understanding performance integration can give insight into predator strategies and diversity that is not apparent otherwise.

The following aims will be addressed in the subsequent chapters to evaluate performance integration and its use during prey capture in fishes:

1. Demonstrate the utility of integration in studies of feeding biomechanics, and that a deeper understanding of ecological differences between species is apparent when integration is considered than when feeding behaviors are considered independent from locomotor behaviors. Two marine sculpin species with divergent locomotor strategies but similar feeding strategies will be compared using 2D kinematics (Chapter 2).
2. Determine new methods to estimate suction accuracy during unconstrained predator-prey interactions, and use this method to assess the relationship between accuracy and predator success. Three species of freshwater centrarchids with divergent locomotor and feeding strategies will be compared using 3D kinematics to predict suction volume and quantify suction accuracy (Chapter 3).
3. Quantify multivariate performance integration and use this method to assess the relationship between integration and accuracy. Multivariate integration will be determined using a novel statistical technique, and assessed in one species of centrarchid from previous work (Chapter 3) capturing divergent prey types. Integration will be related to accuracy to determine the ecological relevance of performance integration for suction-feeding predators (Chapter 4).

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CHAPTER 2:

The Utility of Integration in Feeding Biomechanics

INTRODUCTION

Many vertebrates rely on their locomotor system in order to successfully capture prey, including fishes (Nyberg, 1971; Nemeth, 1997; Rice and Westneat, 2005; Higham, 2007a; Rice, 2008), amphibians (Hoff et al., 1985; Gray and Nishikawa, 1995; Dean, 2003), reptiles (Irschick and Losos, 1998; Alfaro, 2003; Montuelle et al., 2009), birds (Shifferman and Eilam, 2004), and mammals (Dunbar and Badam, 2000; Goldbogen et al., 2007; Kane and Marshall, 2009). Although aquatic animals can capture prey by biting (Bellwood and Choat, 1990), prey capture in fishes commonly incorporates suction and ram (swimming). Whereas ram feeding relies on predator speed to overtake the prey (Liem, 1980; Norton and Brainerd, 1993), suction feeding relies on rapid expansion of the buccal cavity to draw prey towards the predator (Muller et al., 1982; Muller and Osse, 1984). However, suction is useful only over limited distances (Svanbäck et al., 2002; Day et al., 2005) and fishes often combine suction with ram so that most species fall along a continuum between pure suction and pure ram. Metrics have been established to assess species performance in these categories. For example, suction feeding performance can be related to the rate at which the mouth expands (Day et al., 2005; Higham et al., 2006b), but also to the size and timing of maximum gape.

Although suction is a pervasive mechanism for capturing prey in aquatic vertebrates (Lauder, 1985), this strategy can be less efficient when capturing evasive prey (Nyberg,

1971; Norton, 1991; Nemeth, 1997), and ram is often combined with suction to take advantage of prey resources (Liem, 1980; Norton, 1991; Wainwright et al., 2001). Webb (1984b) noted that locomotor behavior can influence prey capture success and feeding mode, and recently, aspects of the locomotor system have been linked to performance of the feeding system (Higham, 2007b). This link between locomotion and feeding indicates that a specific combination of locomotor and feeding behavior is important to successfully capture prey. Integration of these two systems is therefore important for defining feeding strategy.

Studies examining the integration of locomotion and feeding in fishes have typically focused on median and paired fin (MPF) swimmers, including centrarchids (Higham et al., 2005; Higham, 2007a), cichlids (Higham et al., 2007), and labrids (Rice and Westneat, 2005; Collar et al., 2008; Rice, 2008; Rice et al., 2008). Among centrarchids and cichlids, for example, smaller peak gapes are typically correlated with slower swimming speeds and increased strike accuracy (Higham et al., 2006a; Higham et al., 2007). Additionally, pectoral fins can be used not only to decelerate during prey capture (Webb and Skadsen, 1980; Rand and Lauder, 1981; Geerlink, 1987; Higham, 2007a; Rice, 2008), but also for increased maneuverability (Gerstner, 1999; Higham, 2007b). Since locomotor performance during prey capture can influence prey capture success and feeding strategy, understanding how locomotion and feeding are integrated will allow us to better understand the consequences and origins of morphological and functional diversity in fishes that rely on both systems to successfully capture prey. Additionally, understanding how feeding and locomotion perform during the same behavior can add

insight into feeding strategies that may not be apparent by examining feeding performance in isolation.

Marine cottid fishes (Scorpaeniformes: Cottidae) from the Northeastern Pacific Ocean are ideal for studies of predator-prey interactions as they are abundant, morphologically diverse, and exhibit a large degree of diet diversity (Yoshiyama, 1980; Strauss and Fuiman, 1985; Norton, 1991; Norton, 1995). Because of this, they make up an important part of the intertidal and subtidal fauna in the Northeast Pacific. Cottids have been described as locomotor specialists for body and caudal fin (BCF) transient locomotion, with a body form that maximizes thrust. Cottids have also been characterized as benthic specialists, with their pectoral fins adapted for holding position on the substrate in flowing water (Gosline, 1994; Webb et al., 1996). The wide variety of habitat type in cottids, ranging from deep subtidal to tidepools and freshwater systems, results in a wide diversity of morphology that can be correlated to diversity in function. For example, small-mouthed species use suction to capture non-evasive prey items (Norton, 1991; Norton, 1995). However, some species demonstrate inconsistent results, indicating that other potentially important variables, such as the integration of locomotion during prey capture, are important. Additionally, it is unclear whether more atypical cottids demonstrate the link between morphology, feeding strategy, and prey ecology.

Silver-spotted sculpins (*Blepsias cirrhosus*) and tidepool sculpins (*Oligocottus maculosus*) share many similarities that would suggest similarity in feeding strategy. *O. maculosus* can be found sympatrically on the benthos of the same shallow subtidal pelagic habitats as *B. cirrhosus* (Jaenicke et al., 1985) where both species naturally feed

on amphipods (Nakamura, 1971; Norton, 1995; Kolpakov and Dolganova, 2006). This prey type is typical for cottids that rely on suction (Norton, 1991) and indicates that both species should rely on similar feeding modes to capture prey. Additionally, since cottids are specialized for BCF propulsion, both species should display some reliance on ram during prey capture. However, despite these similarities, *B. cirrhosus* are unlike typical cottids in that they inhabit kelp canopies and eelgrass beds, where they actively swim and only occasionally perch on the vegetation (Marliave, 1975). Therefore, a greater reliance on pelagic swimming in *B. cirrhosus* suggests better control over the locomotor system that might allow for tighter integration of the locomotor system with feeding, and would provide insight into the consequences of diversity in cottids that is not apparent from feeding mode alone.

To determine the integration of locomotion and prey capture in divergent cottids, we examined feeding performance and kinematics of *B. cirrhosus* and compared this to more generalized benthic *O. maculosus* for which feeding mode and mouth morphology have been described (Norton, 1991). Although feeding morphology includes components of the hyoid, jaws, and skull, mouth aperture size is an emergent property of these elements that is important when predicting behavior and performance (Wainwright et al., 2001; Higham et al., 2006b), and was used as a proxy of feeding morphology. We addressed the following questions in this study: 1) Do the similarities in mouth morphology, despite differences in ecology, result in convergent feeding kinematics? 2) Are the patterns of integration between the locomotor and feeding systems comparable between *O. maculosus* and *B. cirrhosus*? We predicted that similar feeding morphology will

result in similar feeding kinematics and feeding performance, and that both species will rely on suction to capture prey. We also predicted that the differences in ecology between *B. cirrhosus* and *O. maculosus* will result in differences in the integration of locomotion and feeding, indicating an overall disparity in feeding strategies. An alternative is that, despite differences in ecology, *B. cirrhosus* and *O. maculosus* will exhibit similar integration of the locomotor and feeding systems, resulting in similar feeding strategies to capture prey.

METHODS

Experimental setup

Four *B. cirrhosus* (mean total length: 4.36 ± 0.5 cm) and four *O. maculosus* (mean total length: 6.49 ± 0.5 cm) specimens were seined from seagrass and algae beds on Ross Island (N48°52.4' W125°09.5') and Wizard Island (N48°51.5' W125°09.6') near the Bamfield Marine Sciences Centre (BMSC) in Bamfield, BC, Canada. Juvenile *B. cirrhosus* were chosen to size match with adult *O. maculosus*. In *Clinocottus analis*, ontogenetic changes in feeding kinematics do not occur after larvae settle from the plankton (Cook, 1996). Therefore, although *B. cirrhosus* were juveniles, ontogeny likely contributes little to differences between this species and *O. maculosus*. After collection, specimens were housed in a flow-through sea table maintained at 10°C and starved for 3 days prior to experiments. Specimens were collected under Fisheries and Oceans Canada license XR 80 2010 (TEH), and all collection and experimental procedures were approved by the Animal Care Committee at BMSC.

To determine locomotor and feeding kinematics during prey capture, individuals were transferred to a filming tank (0.5 m x 0.25 m x 0.3 m, ~38L), filled with the same flow-through seawater as the holding tank, and were acclimated from 10 minutes to 1 day, until they appeared calm and willing to feed. During filming, seawater flow was shut off to limit its influence on kinematics, and was re-started at the end of the filming trial to maintain water temperature and reduce animal stress. Individuals were filmed at 500 fps (1080 x 1080 pixels, Photron APX-RS, Photron USA, Inc., San Diego, CA, USA) from the lateral perspective (*B. cirrhosus* 36 trials, *O. maculosus* 23 trials). Gammarid amphipods were collected by hand from an intertidal region near BMSC and were used as prey items. Although these prey items have previously been considered non-evasive (Norton, 1995), they were capable of fast swimming speeds and escape responses. Thus, we consider them evasive with respect to other, non-evasive prey such as polychaete worms, bivalves, echinoderms, and algae (Norton, 1995). Prey items were dropped into the tank once individuals appeared calm and rested on the bottom of the tank (*B. cirrhosus* readily rested on the bottom in the absence of flow). Prey items ranged in size from 3-8mm and larger prey items were fed to larger individuals. Videos were included in the analysis when the predator was visible in lateral view, predator and prey were in focus, the entire length of the predator was visible to determine the initiation of a fast start, and all kinematic landmarks were visible. Trials in which individuals fed from the bottom of the tank were included, as well as missed strikes. However, when a miss was followed by subsequent strikes, only the first strike was included. These criteria resulted

in at least 12 usable sequences (*B. cirrhosus*: 4, 3, 6, and 3 trials for each individual; *O. maculosus*: 2, 3, 2, and 5 trials per individual) for each species.

Data analysis

Sequences were digitized in Matlab (version R2009a, The MathWorks, Natick, MA, USA) using a custom program (Hedrick, 2008), from the initiation of a fast start toward the prey, which was readily apparent in both species after a pause when approaching the prey, until the mouth was closed and jaw protrusion returned to its resting state. Digitized points included: on the prey, 1) the point most distal from the predator, and on the predator, 2) the tip of the premaxilla, 3) the tip of the mandible, 4) the eye, and 5) the distal margin of the caudal fin at the midline (Figure 2.1A). Coordinates were imported into Microsoft Excel 2008 (Microsoft Corporation, Redmond, WA, USA) for further calculations, including: predator total length (TL; linear distance from the tip of the premaxilla to the distal margin of the caudal fin), predator-prey distance (PPD; linear distance from the tip of the premaxilla to the prey) at the start and at mouth opening, predator velocity (linear displacement of the eye over time, smoothed using a quintic spline in Matlab) at the maximum and at peak gape, peak predator acceleration and deceleration (predator velocity displacement over time), peak prey velocity (linear displacement of the prey over time), peak gape (the maximum linear distance between the tips of the premaxilla and mandible), and peak jaw protrusion (the maximum linear distance from the tip of the premaxilla to the eye). Peak cranial elevation (displacement of the angle formed by the tip of the premaxilla and the bases of the first

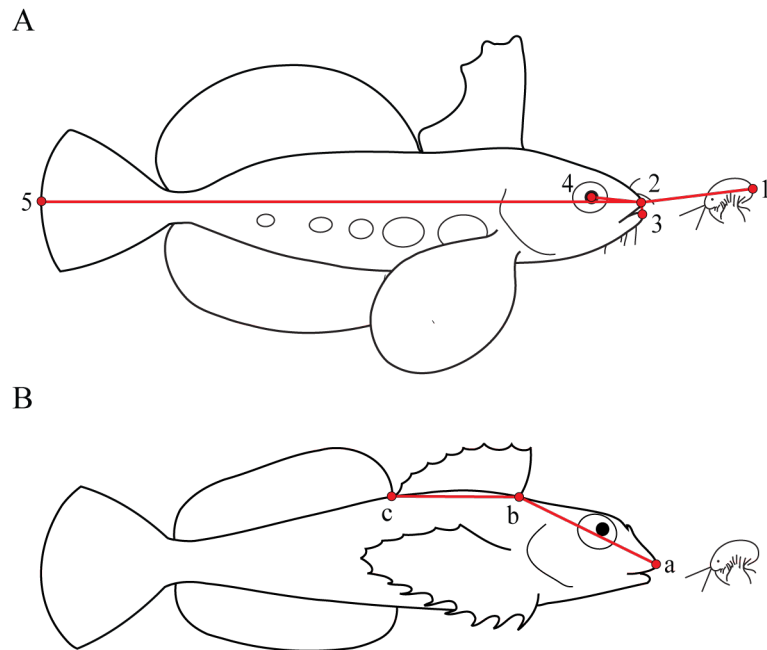


Figure 2.1 Representative diagrams of A) digitized anatomical landmarks and kinematic measurements shown on *Blepsias cirrhosus* and B) static measurements shown on *Oligocottus maculosus*. All measurements were taken from each species. Diagrams are scaled to the same total length to show relative differences in body morphology between species. Kinematic landmarks were used to calculate predator total length (points 2 to 5), predator-prey distance (points 1 to 2), gape (points 2 to 3), and upper jaw protrusion (points 2 to 4). Cranial elevation (angle abc) was calculated as the difference between values at the start and the maximum. See the text for a description of additional calculated variables.

spine of the first and second dorsal fins; Figure 2.1B) was calculated in Image J (version 1.43r, NIH, Bethesda, MD, USA) at the frames of fast start initiation and maximal displacement (determined visually). Finally, timing of all kinematic events was recorded, in addition to time of fin abduction, time of mouth opening, time of prey capture (when the prey crossed the boundary created by the tips of the premaxilla and the mandible), time to peak gape (TTPG; duration from mouth opening to peak gape), and total duration (from fast start initiation to peak gape). Timing (except for total duration)

was normalized to ms before or after peak gape, with events occurring before peak gape having negative values. To determine if species displayed similar mouth sizes, and therefore, feeding morphology, mouth area was calculated by assuming a circular aperture shape at peak gape, where peak gape distance represents the diameter.

Statistical analysis

Statistics were performed in JMP (version 8.0.2, SAS Institute Inc., Cary, NC, USA). Mouth area was log transformed and regressed against log transformed total length, similar to Norton (1991). A regression line was fit to the combined data for both species. Each species was then constrained to the combined regression equation, and significance of the fit was determined. For the remaining analyses, length measurements (PPD, peak gape, jaw protrusion), velocities (predator and prey), and accelerations were scaled to TL, although data reported below are unscaled values.

Only *B. cirrhosus* had enough missed strikes (6 out of 17 usable sequences; 1-2 misses per individual) to determine kinematic differences between successful and unsuccessful strikes. Missed strikes were not significantly different than captured strikes for individual means of all variables (t-tests, $p > 0.2131$). Similarly, only *B. cirrhosus* captured prey from the bottom (5 out of 17 sequences; 0-3 bottom strikes per individual). However, strikes near the bottom occurred during forward movement (and were not directed at the bottom) and individual means were not significantly different from strikes in the water column (t-tests, $p > 0.1129$) except for PPD at mouth opening (t-test, $p = 0.0157$). Therefore, data were pooled for further analyses.

To distinguish differences between species, all dependent variables were assessed using 2-way analyses of variance (ANOVAs) with species (fixed factor) and individual (random factor nested within species) as independent variables. Results were Bonferroni corrected (Rice, 1989) and resulting critical values were $p < 0.004$ for kinematic and $p < 0.005$ for timing variables. Sequential Bonferroni corrections were not used because these are more conservative and can inflate Type II error (Moran, 2003). Additionally, coefficient of variation (CV) for all variables was calculated for all individuals, and significance was tested using a t-test on species to determine differences in stereotypy (Wainwright et al., 2008). Results were also Bonferroni corrected with the same critical values. Finally, Pearson correlations were used to indicate locomotor variables that were correlated with feeding kinematics to determine the integration between locomotion and feeding.

To explore the variability of and visually summarize feeding and locomotor kinematics, a principal components analysis (PCA) was run using the correlation matrix on the mean of all kinematic variables for each individual. This was done to maintain the assumption of independent samples. Variables were correlated to the PC scores for each PC axis and significance was determined using a Pearson correlation. A t-test on PC scores was used to determine if species differed significantly in placement along each PC axis.

RESULTS

Both *B. cirrhosus* and *O. maculosus* captured amphipod prey in the water column using a fast start to approach the prey and then a combination of suction and ram for capture. Individuals oriented to the prey, sometimes approaching with short bursts of swimming, and paused before beginning the fast start. Strikes were initiated from approximately 1.8 cm and 2.6 cm away from the prey for *B. cirrhosus* and *O. maculosus*, respectively, which was $0.41 \cdot TL$ for both species. Both species rested on the bottom of the tank and entered the water column to capture swimming prey; however, *B. cirrhosus* readily captured prey from any depth (including the bottom of the tank), whereas *O. maculosus* always captured prey almost immediately after being introduced at the top of the water column, and rapidly returned to the bottom of the tank. Capture success was greater for *O. maculosus* (91% in 23 trials) than *B. cirrhosus* (72% in 36 trials). Feeding morphology (mouth size) and kinematics were similar between species. The relationship between mouth area and total length shows that both species have similar mouth areas for their given sizes (Figure 2.2). Species were constrained to a common regression line ($y = 1.28x - 0.80$), which was significant for both species (*B. cirrhosus*: $t = 58.2, p < 0.0001$; *O. maculosus*: $t = 56.95, p < 0.0001$). Elevation of the cranium was $18.9 \pm 2.33^\circ$ and $13.3 \pm 1.19^\circ$ for *B. cirrhosus* and *O. maculosus*, respectively. Additionally, the jaws were protruded to approximately 3% of TL in both species. Although peak gape appeared smaller for *B. cirrhosus* (*B. cirrhosus*: 0.49 ± 0.02 cm; *O. maculosus*: 0.63 ± 0.03 cm), differences in cranial elevation, protrusion, and gape were not significant (ANOVAs, $p > 0.01$).

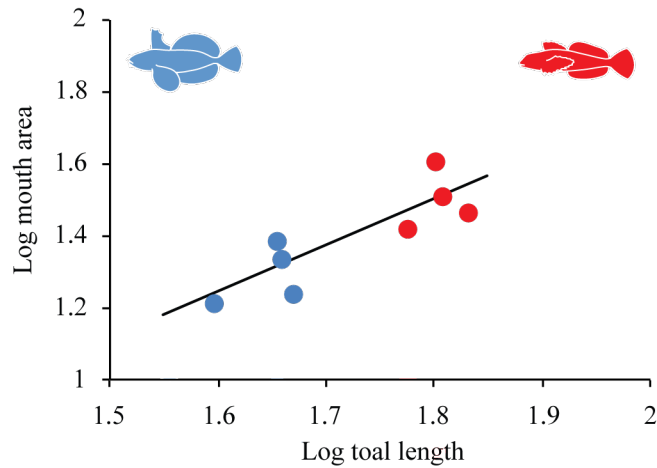


Figure 2.2 relationship between log transformed mouth area (mm^2) vs. log transformed total length (mm) for *B. cirrhosus* (blue) and *O. maculosus* (red). Mouth area was calculated by assuming that peak gape represents the diameter of a circle. Both species fall on a common regression line ($y = 1.28x - 0.80$), indicating that both should employ similar suction feeding strategies to capture prey (Norton, 1991).

Univariate tests on each variable showed that both species relied on degrees of ram and suction during prey capture that resulted in overall similar feeding modes. Peak velocity (*B. cirrhosus* 25.5 ± 2.67 ; *O. maculosus* 30.3 ± 2.74) and velocity at peak gape (*B. cirrhosus* $24.4 \pm 2.63 \text{ cm s}^{-1}$; *O. maculosus* $26.4 \pm 2.28 \text{ cm s}^{-1}$) were comparable between the two species. Both species also initiated mouth opening at 0.77 ± 0.09 and $1.27 \pm 0.16 \text{ cm}$ from the prey for *B. cirrhosus* and *O. maculosus*, respectively. Peak prey velocities were 67.4 ± 15.2 and $93.2 \pm 10.7 \text{ cm s}^{-1}$ for *B. cirrhosus* and *O. maculosus*, respectively. None of these variables were significantly different between species (Table 2.1; ANOVAs, $p > 0.008$). Although time of prey capture indicated that *B. cirrhosus* captured prey later in the gape cycle, and closer to peak gape, than *O. maculosus* (Table 2.1), when this value was scaled to a percentage of TTPG, no significant differences were found (ANOVA, $p = 0.10$).

Table 2.1 Mean \pm s.e.m. for select kinematic variables after standardization to TL

Variable	<i>B. cirrhosus</i> (17)	<i>O. maculosus</i> (12)	Species	Individual
Total length (cm)	4.36 \pm 0.11	6.49 \pm 0.13	0.000*	0.003*
Peak velocity (BL s ⁻¹)	5.91 \pm 0.63	4.67 \pm 0.41	0.049	0.007
Time of peak velocity, normalized (ms)	-3.76 \pm 1.55	-19.00 \pm 4.65	0.000*	0.000*
Peak acceleration (BL s ⁻²)	155.61 \pm 18.32	63.85 \pm 6.32	0.000*	0.281
Time of peak acceleration, normalized (ms)	-16.12 \pm 2.19	-56.17 \pm 6.86	0.000*	0.004*
Peak deceleration (BL s ⁻²)	-190.76 \pm 23.56	-61.74 \pm 7.02	0.000*	0.011
Time of peak deceleration, normalized (ms)	12.59 \pm 1.61	11.50 \pm 3.82	0.934	0.655
Peak prey velocity (captures; BL s ⁻¹)	20.27 \pm 4.32	15.55 \pm 1.34	0.240	0.285
Time of peak prey velocity, normalized (ms)	0.55 \pm 1.40	-3.45 \pm 2.07	0.059	0.002*
Time of mouth opening, normalized (ms)	-19.65 \pm 1.89	-31.83 \pm 2.32	0.000*	0.002*
PPD opening/TL	0.18 \pm 0.02	0.20 \pm 0.03	0.192	0.093
Time of prey capture, normalized (ms)	-4.00 \pm 1.13	-10.36 \pm 1.76	0.001*	0.056
Peak gape/TL	0.11 \pm 0.00	0.10 \pm 0.00	0.012	0.077
Time to peak gape (ms)	19.65 \pm 1.89	31.83 \pm 2.32	0.000*	0.002*
Velocity at peak gape (BL s ⁻¹)	5.64 \pm 0.61	4.05 \pm 0.33	0.008	0.011
Total duration (ms)	128.82 \pm 17.15	98.83 \pm 10.63	0.044	0.000*

Values are mean \pm s.e.m. with number of trials in parentheses.

P-values from 2-way ANOVA shown for species and individual effects.

* Significant differences at $p < 0.004$ (kinematic variables) or $p < 0.005$ (timing variables)

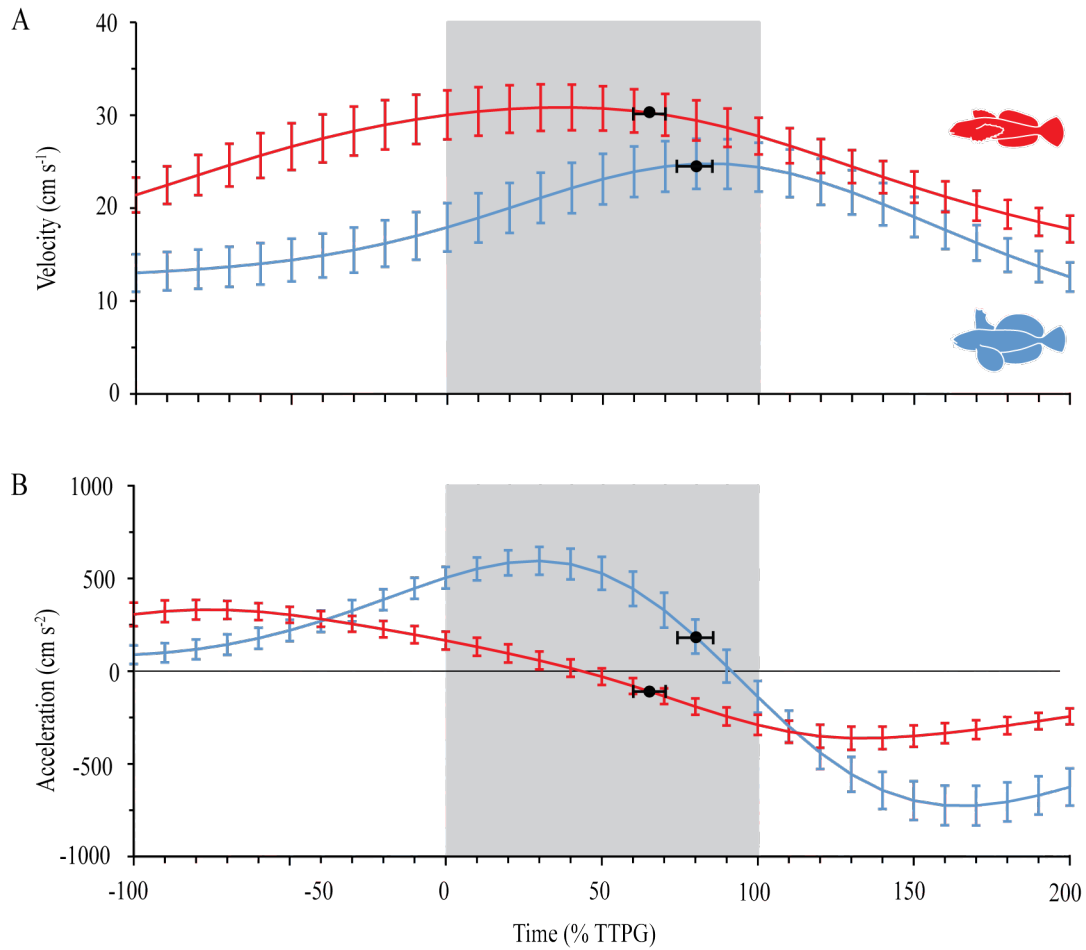


Figure 2.3 Mean \pm s.e.m. A) velocity and B) acceleration of *B. cirrhosus* (blue) and *O. maculosus* (red) while feeding on amphipod prey. Timing was scaled to %TTPG and trials were interpolated to 31 points from 100% TTPG before mouth opening to 100% TTPG after peak gape. Gray shading represents TTPG, bounded by time of mouth opening and peak gape. Large black dots on each trace indicate mean time of prey capture \pm s.e.m. for each species. While *B. cirrhosus* relies on rapid acceleration to increase velocity and capture prey near peak gape, *O. maculosus* accelerates more slowly before mouth opening and decelerates as prey is captured.

Maximum body acceleration in *B. cirrhosus* occurs during mouth opening, and forward velocity was maintained through prey capture, with deceleration beginning prior to the time of peak gape (Figure 2.3A). In contrast, *O. maculosus* accelerated during the fast start, before mouth opening, slowing velocity and beginning deceleration prior to the time of prey capture (Figure 2.3B). Both species continued deceleration until a peak after the time of peak gape. Although peak body velocity was not significantly different between species, it occurred less than 4 ms before peak gape in *B. cirrhosus*, but 19 ms before peak gape in *O. maculosus* (ANOVA, $p = 0.049$; Table 2.1). Additionally, peak body acceleration was significantly greater for *B. cirrhosus* ($675.1 \pm 75.8 \text{ cm s}^{-2}$; Figure 1.3B) than for *O. maculosus* ($416.3 \pm 43.2 \text{ cm s}^{-2}$; ANOVA, $p < 0.001$). However, both species reach peak deceleration approximately 12 ms after peak gape, aided by pectoral fin protraction, with maximum pectoral fin abduction occurring more than 30 ms after peak gape. Feeding and locomotor kinematics were highly variable for both species, and both species exhibited comparable levels of stereotypy (measured using coefficient of variation; t-tests, $p > 0.05$).

As indicated both by correlations between locomotor and feeding variables, integration between locomotion and feeding was more apparent in *B. cirrhosus* than *O. maculosus*. For *B. cirrhosus* PPD at mouth opening (Figure 2.4A), approach velocity (Figure 2.4C), and absolute value of deceleration (Figure 2.4E) were positively correlated with peak gape (Pearson correlations, $r > 0.52$, $p < 0.03$). For *O. maculosus* PPD at the start and at mouth opening were the only variables correlated with peak gape (Figure

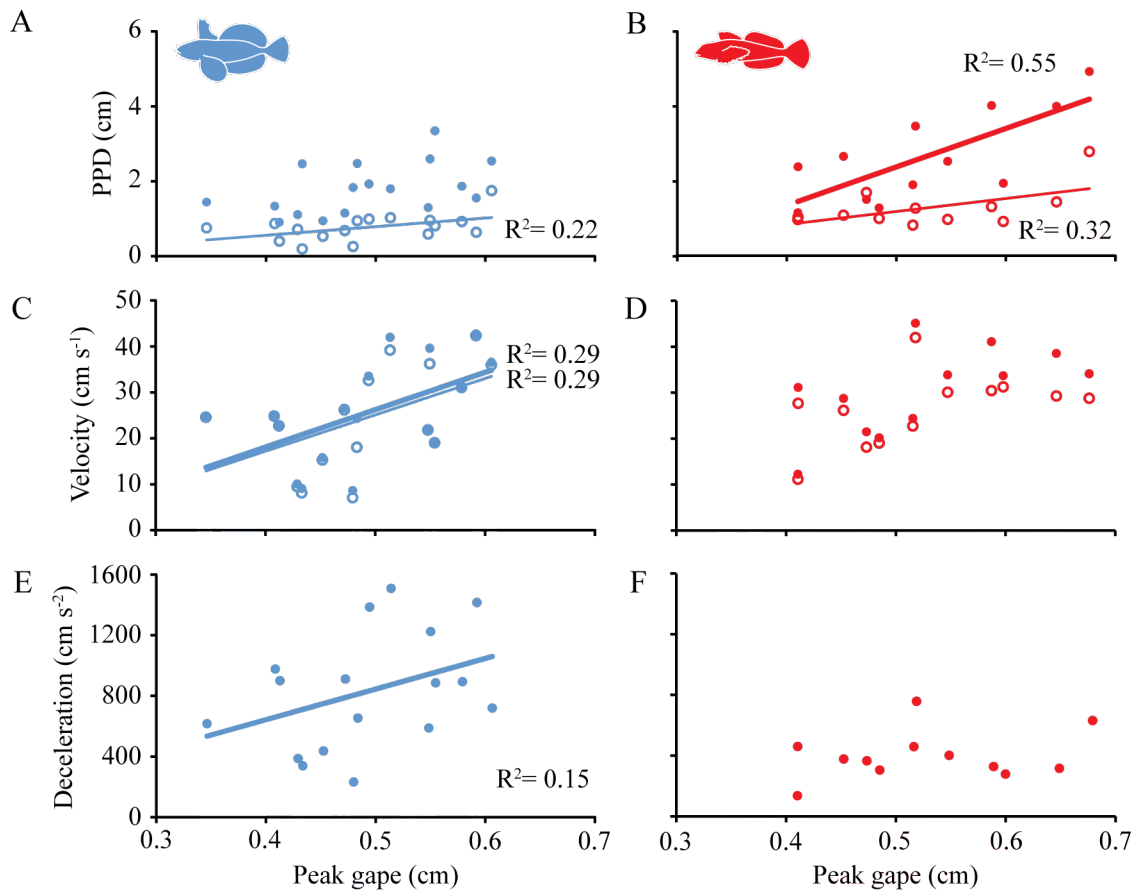


Figure 2.4 Integration of locomotor variables with peak gape for *B. cirrhosus* (blue) and *O. maculosus* (red). Points are shown on unstandardized scales for each trial for both species, but only significant correlations from standardized (to total length) data are indicated. Regression lines and associated R² values are shown for unstandardized data. A-B) Predator-prey distance (PPD) at the start (solid circles and thick lines) and at mouth opening (open circles and thin lines); C-D) peak velocity (solid circles and thick lines) and velocity at peak gape (open circles and thin lines); E-F) absolute value of peak deceleration. PPD, strike velocity, and deceleration are correlated to peak gape in *B. cirrhosus*, whereas only PPD is correlated to peak gape in *O. maculosus*, indicating a greater contribution of the locomotor system to the feeding strategy of *B. cirrhosus*.

2.4B; Pearson correlation, $r = 0.60$, $p = 0.04$). Integration was not observed between predator approach velocity (Figure 2.4D) or predator deceleration (Figure 2.4F) in *O. maculosus*.

Species were distinct in multivariate space, and were separated by two axes of variation (Figure 2.5) that explained 60.7% of the total variance between species (PC1 32.8%; PC2 27.9%). PC1 was positively correlated with peak acceleration, time of peak acceleration, time of peak velocity, time of mouth opening, time of prey capture, and peak deceleration (Table 2.2; Pearson correlations, $r > 0.72$, $p < 0.044$) and was negatively correlated with TTPG (Table 2.2; Pearson correlation, $r = -0.93$, $p = 0.0009$). This axis represents differences in magnitude of acceleration and deceleration, and the timing of locomotor and feeding performance measures (occurrence prior to or near the time of peak gape). PC2 was only positively correlated with many of the remaining variables, including starting PPD, peak velocity, peak gape, velocity at peak gape, and peak cranial elevation (Table 2.2; Pearson correlations, $r > 0.74$, $p < 0.036$). These variables summarize differences in feeding mode, and therefore, PC2 represents an axis describing degrees of ram and suction use. *B. cirrhosus* and *O. maculosus* were significantly different for PC1 (integration; t -test, $t = -3.81$, $p = 0.01$), but not PC2 (feeding mode; t -test, $t = -0.47$, $p = 0.66$).

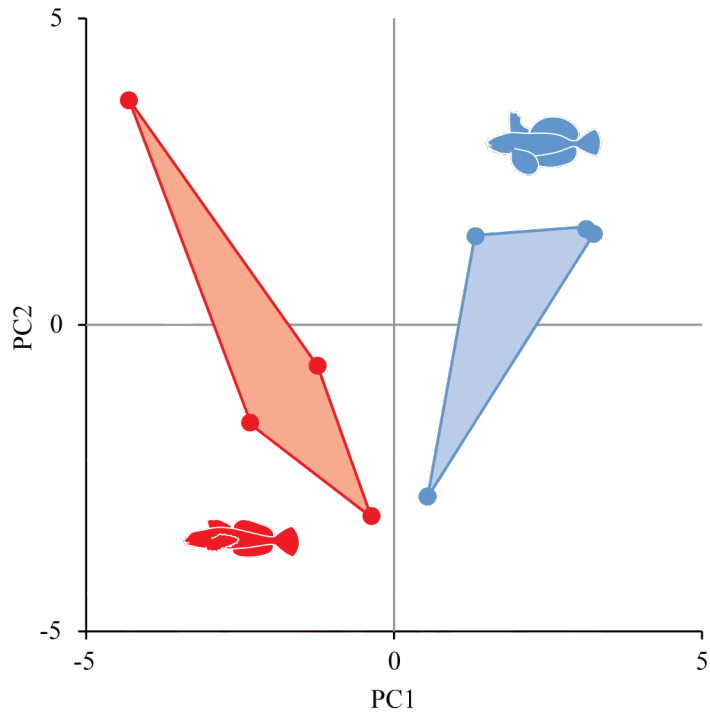


Figure 2.5 Principal component (PC) scores for individuals of *B. cirrhosus* (blue) and *O. maculosus* (red) plotted in PC space. For kinematic variables that correlated with each axis, see Table 2.2. Species separate primarily along the (PC1) axis.

Table 2.2 Variable correlations with principal component axes

Variable	PC1	PC2
PPD at start	-0.37	0.81*
Peak acceleration	0.79*	0.55
Time of peak acceleration	0.92*	-0.23
Peak velocity	0.40	0.88*
Time of peak velocity	0.80*	-0.58
PPD at mouth opening	-0.34	0.23
Time of mouth opening	0.93*	-0.20
Peak prey velocity	0.31	-0.18
Time of peak prey velocity	0.50	-0.54
Time of prey capture	0.72*	-0.31
Peak gape	0.37	0.74*
Velocity at peak gape	0.61	0.74*
Time to peak gape	-0.93*	0.20
Peak cranial elevation	0.38	0.74*
Time of max cranial elevation	0.28	-0.16
Peak protrusion	0.21	0.40
Time of max fin abduction	0.37	-0.27
Peak deceleration	0.88*	0.43
Time of peak deceleration	0.21	-0.50
Total duration	-0.18	0.00

* Significant correlations at $p < 0.05$; Pearson correlation between original variables and principal component scores for axes 1 (PC1) and 2 (PC2).

DISCUSSION

Morphological variation is often the basis for studies addressing the link between form and function. However, morphology does not always provide a direct link to function, as structures can perform multiple functions and, in some cases, structures that appear different can accomplish similar functions (Wainwright, 2007). Our study focused on two cottid species that exhibit comparable feeding morphology (mouth size) and kinematics, but exhibit variation in locomotor performance, locomotor integration with feeding, and therefore, feeding strategy. For both *B. cirrhosus* and *O. maculosus*, mouth size predicts feeding kinematics involving suction. However, *B. cirrhosus* achieved greater acceleration during prey capture, and displayed a tighter integration of locomotor and feeding variables. Our prediction that the feeding strategy of divergent suction feeding cottids would be reflected in levels of integration of locomotion and feeding is supported. However, locomotor morphology was not quantified in this study, and it is possible that differences in locomotor kinematics and performance during prey capture result from differences in locomotor morphology, an area that remains to be explored.

This is the first study to address the integration of locomotion and feeding in cottids, which are body and caudal fin (BCF) transient locomotor specialists (Webb, 1984b). Studies of locomotion and feeding in BCF specialists have not addressed the integration of these behaviors, and have not included predator velocity and acceleration profiles when determining differences between species (Webb and Skadsen, 1980; Rand and Lauder, 1981; Webb, 1984a; Harper and Blake, 1991; Porter and Motta, 2004). Our

study indicates that multidimensional analyses of locomotor and feeding performance can provide insight into differences between species that are not apparent otherwise.

Therefore, future studies that aim to explain patterns of feeding and locomotor diversity in fishes should include acceleration profiles that not only describe the magnitude of velocity and acceleration, but also how these variables change with time, and how they are related to the timing of other variables, such as prey capture. This type of analysis has been valuable in inferring patterns of diversity in divergent marine cottids with similar feeding morphology and kinematics.

The use of suction during prey capture

Sculpins in this study displayed similar mouth size that was consistent with previous descriptions of suction feeding cottids. For several species of cottids, Norton (1991) determined that the relationship between mouth area and body length could accurately predict feeding mode. Differences in mouth area were used as a proxy of overall morphological feeding differences, and primarily separated species into large-mouthed ram feeders, and small-mouthed suction feeders (Figure 1 in Norton, 1991). When *B. cirrhosus* and *O. maculosus* are plotted in a similar manner (Figure 2.2), both species fall on the same regression line. In morphological space, these species occur in an area also occupied by other small-mouthed suction feeding cottids, including *O. maculosus* (Norton, 1991). Therefore, both species in this study exhibit similar mouth morphology (mouth size) that is consistent with the ability for suction feeding during prey capture.

Similarities in prey velocity during prey capture indicated not only that predator strategies were not affected by prey movement, but also that suction performance was similar between species. However, suction performance may have been greater in *O. maculosus* than in *B. cirrhosus*. Suction performance can be estimated by prey velocity at prey capture, and in this study, *O. maculosus* was able to ingest prey at a greater velocity than *B. cirrhosus*, although this difference wasn't significant. It is possible that differences in suction generation performance that weren't quantified in this study (fluid flow velocity, suction force, etc.) could have contributed to increased suction performance in *O. maculosus*. For example, it is not known whether peak fluid speed occurs at the time of peak gape, as is the case for other teleosts (Day et al., 2005; Higham et al., 2006b). Future work utilizing digital particle image velocimetry to assess the hydrodynamics of suction feeding in cottids will allow a more accurate interpretation of the patterns of prey capture behavior observed in this study.

Locomotor and feeding strategies

Differences in fast start performance during prey capture between *B. cirrhosus* and *O. maculosus* are associated with differences in microhabitat (benthic vs. pelagic). The similarity in results from both univariate analyses and the PCA indicates that the feeding strategies of each species are prominent and defining. Whereas *O. maculosus* adopts a strategy of early acceleration, reaching peak velocity and beginning to decelerate as the prey is captured, *B. cirrhosus* accelerates just prior to prey capture, resulting in peak velocity occurring at or just after prey capture, and deceleration beginning at peak gape

(Figure 2.3). This strategy of *B. cirrhosus* is similar to *Micropterus salmoides* and *Lepomis macrochirus*, which both accelerate prior to peak gape and through prey capture (Higham, 2007a). The alternative strategy of *O. maculosus* may be due to its benthic behavior compared to the pelagic behavior of the other species. *O. maculosus* typically turns around after prey capture to immediately return to the bottom of the aquarium. Therefore, deceleration through prey capture may facilitate turning maneuverability, and escape to the protection of rocks and vegetation after prey capture. In shallow tidepool habitats, *O. maculosus* are best protected from predators when sitting still on the benthos among vegetation. Therefore, this species likely relies on a strategy that minimizes exposure to predators. Pelagic species, such as *B. cirrhosus*, *M. salmoides*, and *L. macrochirus* do not have this constraint, and therefore display a feeding strategy that allows them to overrun prey while remaining in the water column.

These differences in feeding strategies between species might be responsible for the greater capture success rates observed in *O. maculosus* compared to *B. cirrhosus*. Differences in acceleration profiles reflect differences in velocity at prey capture such that velocity is increasing for *B. cirrhosus* but decreasing for *O. maculosus* as the prey enters the mouth. Therefore, *O. maculosus* is likely better able to aim at prey since accuracy increases with decreased velocity (Higham et al., 2006a). In the current study, missed strikes qualitatively appeared to be the result of poor aim, and not prey behavior since escape responses were typically initiated after a failed strike. Therefore, increased accuracy in *O. maculosus* likely results in greater capture success.

The feeding strategies of cottids in this study differed primarily in the reliance on integration. Compared to *O. maculosus*, *B. cirrhosus* exhibited a greater degree of integration between the locomotor and feeding systems during prey capture (Figure 2.4). However, this does not rule out the possibility that benthic station-holding *O. maculosus* also rely on some degree of integration of the locomotor system with feeding. For example, similar velocities were used by both species to approach the prey and both species employed pectoral fin protraction to decelerate after prey capture. Future work investigating the detailed kinematics of the locomotor system during prey capture might reveal a level of integration not found in the current study.

Both species of cottids in this study relied on fast starts to capture prey. This behavior is well documented in several cottids (Norton, 1991; Cook, 1996) and esocid pikes (Webb and Skadsen, 1980; Rand and Lauder, 1981; Harper and Blake, 1991), and supports the hypothesis that the sculpin and pike body forms are used to generate rapid acceleration during prey capture as a means to ambush prey (Webb, 1984b). However, cottid adaptations for BCF transient propulsion are drag minimizing, unlike the thrust maximizing body form of esocids (Webb, 1984b), and this is reflected by greater velocities and accelerations during prey captures and escapes in esocids than cottids. During feeding fast starts, *Esox lucius* accelerates between 174-344 BL/s², and reaches velocities of 8.5 BL/s (Harper and Blake, 1991). In contrast, cottids in this study performed similar to the cottid *Myoxocephalus scorpius*, lunging at prey with a velocity of approximately 5 BL/s and accelerating at only 91 BL/s² (Beddow et al., 1995). Although cottid feeding fast start performance differs from esocids in magnitude, *B.*

cirrhosus utilizes approximately 63% of maximal escape performance during feeding strikes (S. Kawano, personal communication), which is similar to the esocid *E. lucius*, which reaches approximately 66% of maximal escape performance (Harper and Blake, 1991). The ability of *B. cirrhosus* to closely match feeding and escape performance, similar to esocids, indicates that the feeding strategy is similar between taxa even though the magnitude of performance is lower. Alternatively, *M. scorpius* utilizes 54% of maximal performance during feeding strikes (James and Johnston, 1998; Temple and Johnston, 1998). Given the differences previously described between *B. cirrhosus* and *O. maculosus*, as well as the similarity in benthic ecology and body form of *O. maculosus* and *M. scorpius*, it can be predicted that *O. maculosus* might rely on a feeding strategy similar to *M. Scorpius* where a lesser degree of maximal escape performance is utilized during feeding fast starts. However, this hypothesis has not yet been tested.

Implications for diversity

It has been shown that freshwater cottids can display variation in morphology based on habitat, resulting in forms more or less morphologically adapted for benthic station-holding (Kerfoot and Schaefer, 2006). Our study provides evidence that variation in ecology can contribute to variation in locomotor performance that is then reflected in the integration of locomotion and feeding and overall feeding strategy. This relationship between ecology and function is likely linked by a relationship to morphology. For example, *O. maculosus* pectoral fins are specialized for benthic station holding (Gosline, 1994), and a trade-off in pectoral fin function and performance might limit the

movements of the pectoral fins across behaviors. Specifically, the stabilizing actions of the pectoral fins during swimming behaviors could be compromised, resulting in their observed increased reliance on deceleration at prey capture to moderate this constraint. Alternatively, *B. cirrhosus* pectoral fins are released from station-holding morphological constraints, allowing the fins to evolve further integration of locomotion and feeding, as was observed in this study. Further analyses of the effects of habitat on morphology and function in cottids would give a better indication of whether these functional trade-offs are generally apparent across cottids, or whether they are specific to the two species studied here.

Recent studies have supported the idea that deep subtidal cottids like *B. cirrhosus* represent an ancestral form, whereas cottids in shallower or freshwater habitats like *O. maculosus* represent more derived forms (Ramon and Knope, 2008; Mandic et al., 2009). If the active, pelagic lifestyle of *B. cirrhosus* (Marliave, 1975) is representative of ancestral cottids, it is possible that swimming performance has been ancestrally selected for, facilitating integration during prey capture in more basal species. In cichlids, ram speed and peak gape are evolutionarily correlated (Higham et al., 2007). However, this relationship was only observed for *B. cirrhosus* in this study. The lack of a relationship in more derived *O. maculosus* indicates that ram speed may not be evolutionarily correlated to peak gape in cottids, and that integration between locomotion and feeding has been lost in more derived lineages. It is possible that in derived shallow water forms the need for integration is superceded by demands from the habitat. This is likely a common pattern among vertebrates, but one that is apparent in cottids due to their

specialized benthic ecology. These hypotheses should be tested within a phylogenetic framework to determine how changes in habitat, morphology, and performance are correlated in cottids.

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CHAPTER 3:

The Influence of Approach Accuracy on Predator Success

INTRODUCTION

The ability to capture prey is critical for survival in predatory animals. Capture strategies vary depending on specializations in predator and prey and the outcome is determined by performance and behavior of both the predator and prey (Britton and Jones, 1999; Shifferman and Eilam, 2004; Walker et al., 2005; Combes et al., 2013; Stewart et al., 2013). For predators, a large component of success is likely due to strike accuracy, or the ability to correctly position and time a strike (Drost, 1987). In this way accuracy is the link between feeding performance (how well predators perform feeding behaviors) and predator success (whether or not prey are captured), and acts as an ecologically relevant measure of capture performance (Irschick et al., 2008). However, despite the importance of accuracy to predator strategies, our understanding of the determinants of predator success is poor, particularly in suction-feeding fishes (but see Drost, 1987; Nauwelaerts et al., 2008; Holzman et al., 2012; Maie et al., 2014).

Suction is a widespread predatory strategy employed by fishes (Alexander, 1967; Lauder, 1985; Norton and Brainerd, 1993; Wainwright et al., 2001) and accuracy is particularly important for these predators, but quantifying accuracy has been difficult. Suction is generated by rapidly expanding the mouth cavity, creating a pressure gradient that draws water and prey into the mouth (Alexander, 1967; Weihs, 1980; Muller and Osse, 1984; Day et al., 2005; Day et al., 2007; Holzman et al., 2007). Because suction

force decays rapidly with distance from the mouth (Ferry-Graham et al., 2003; Day et al., 2005), predators must accurately position and time the suction field to successfully entrain prey (Ferry-Graham et al., 2003; Holzman et al., 2007). The term ‘accuracy’ has been used synonymously with capture success, where predators with greater success are assumed to be more accurate (Nyberg, 1971; Webb and Skadsen, 1980; Norton, 1991; Coughlin, 1994; McLaughlin et al., 2000; Rincón et al., 2007). However, a successful prey capture event depends on several factors related to both the predator and prey (Holzman et al., 2012), and accuracy may not be directly related to success. Another more direct method relates predator and prey position linearly (Drost, 1987; Coughlin, 1991; Holzman et al., 2007). However, it may not be relevant for suction feeding fishes, where predators rely on a volume of water positioned anterior to their jaws to capture prey (Ferry-Graham et al., 2003; Day et al., 2005; Higham et al., 2005; Higham et al., 2006; Day et al., 2007), rather than their jaws directly. Therefore, predator accuracy should instead be quantified relative to the ingested volume of water (IVW) (Higham et al., 2005; Higham et al., 2006). This requires fluid visualization techniques such as Digital Particle Image Velocimetry (DPIV) that can be labor intensive, inhibiting large-scale analyses of suction accuracy and understanding its importance for predators.

The IVW contains all particles of water that enter the mouth cavity during a feeding event (Van Leeuwen, 1984). The ability to define the IVW is possible because suction forces decay rapidly with distance from the mouth (Ferry-Graham et al., 2003; Day et al., 2005), limiting the number of particles that are ingested. Ingested particles are tracked back to the frame of mouth opening and encircled, defining the volume within which all

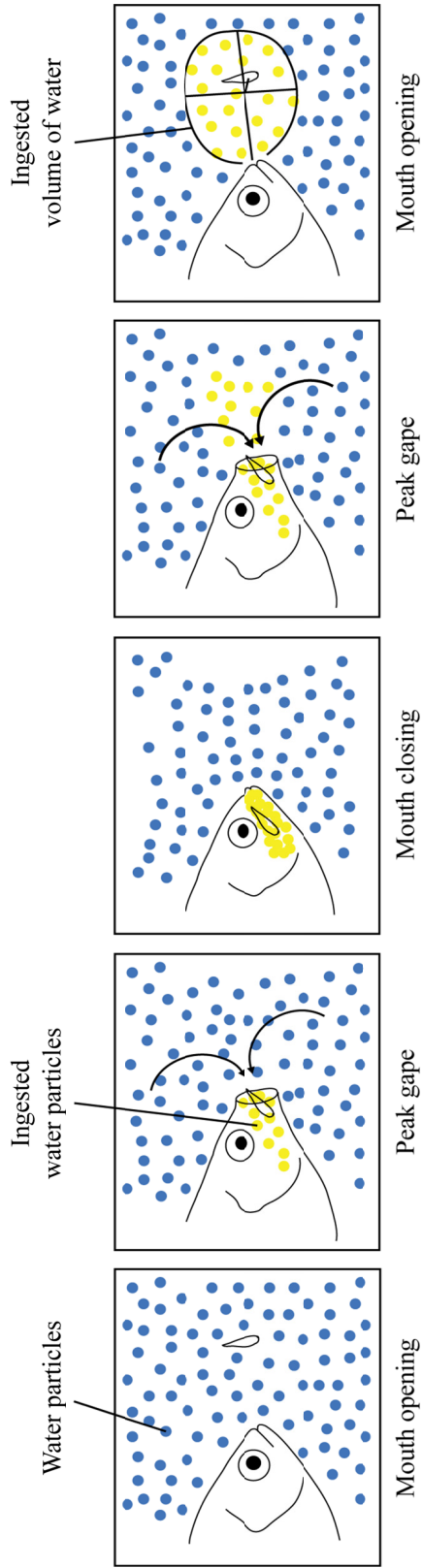


Figure 3.1 Schematic demonstrating how the ingested volume of water is quantified from digital particle image velocimetry (DPIV) data. Particles that enter the mouth between opening and closing are tracked, then traced backwards to the frame of mouth opening and encircled. Accuracy is then calculated by relating the prey position to the center of this volume.

particles are ingested (Higham et al., 2005; Higham et al., 2006; Nauwelaerts et al., 2008). In this way the ingested volume represents a summation through time but is visualized spatially in the frame of mouth opening (Figure 3.1). Suction accuracy is then quantified by relating prey position to the center of the ingested volume (Figure 3.2; (Higham et al., 2006)). As an effect of particle summation, the length axis represents time since the first particle ingested is closest to the predator and the last particle is farthest (relative to the start position). Additionally, the position of maximum height corresponds to the timing of peak gape when suction force is the strongest (Day et al., 2005) and 50% of the total volume has been ingested (Higham et al., 2006). Therefore, the greatest suction accuracy should be experienced when prey are located closer to the center of the IVW because this is the point where suction is strongest and because it maximizes the distance to the edge of the volume, limiting the possibility for prey to escape the volume (Van Leeuwen, 1984). By quantifying suction accuracy relative to the center of the IVW, accuracy reflects both the correct timing and position of the IVW relative to the prey.

This method of quantifying accuracy has helped describe performance differences within and between species (Higham et al., 2005; Higham et al., 2006; Nauwelaerts et al., 2008). For example, although *Lepomis macrochirus* (bluegill sunfish) is more accurate, *Micropterus salmoides* (largemouth bass) generates a larger ingested volume that may be beneficial for capturing evasive prey (Higham et al., 2006). Additionally, successful strikes in *Chiloscyllium plagiosum* (white-spotted bamboo sharks) occurred with increased accuracy (Nauwelaerts et al., 2008). However, DPIV limits the variation in

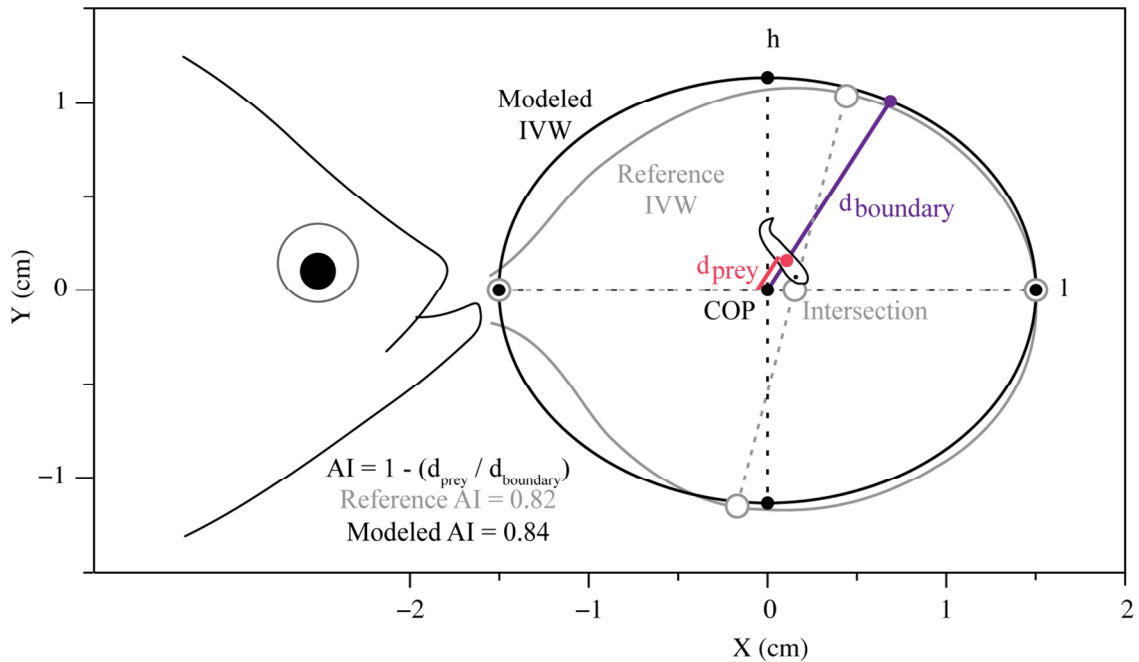


Figure 3.2 Comparison of reference and modeled IVW shapes. Open gray circles show the top, left, bottom, and right boundary anchor points of an actual IVW trace from the reference dataset (Higham et al., 2005), and the connecting gray line shows a typical outline of an IVW anchored on these points. Using the length (l) and height (h) determined from these anchor points, the IVW was modeled as a 2D ellipse (solid line and points). Due to this estimation, the center of the parcel (COP, center of the ellipse) was not always located at the intersection of l and h . Accuracy (AI) was calculated as the ratio of the distance from the center of the modeled IVW to the prey center of mass (offset red line, d_p) relative to the distance from the COP to the boundary of the ellipse, through the prey center of mass (purple line, d_b), subtracted from one. Predator not drawn to scale. The modeled IVW provides a good approximation of the reference IVW.

both predator accuracy and success because it requires precisely controlled positions of predator and prey so that the feeding event is captured within the laser sheet (Higham et al., 2005; Higham et al., 2006; Nauwelaerts et al., 2008). This restrictive environment is not representative of natural conditions, and for suction accuracy to be a more applicable performance measure, it is necessary to define a method for estimating shape parameters

of the IVW based on non-invasive measurements such as 3D kinematics. Since simple kinematics such as gape, time to peak gape, and predator ram speed are indicators of suction performance (Sanford and Wainwright; Holzman et al., 2007), and since the IVW encompasses the particles ingested as a result of suction performance (Van Leeuwen, 1984; Higham et al., 2005), these kinematic parameters can also be expected to predict IVW shape parameters such as length and height. We use centrarchid fishes (freshwater sunfishes and basses) to construct a regression-based model of IVW shape to quantify suction accuracy during more natural predator-prey encounters than what can be achieved by using DPIV-based methods.

Centrarchids are a model system for understanding not only the mechanics of suction (Sanford and Wainwright, 2002; Ferry-Graham et al., 2003; Day et al., 2005; Higham et al., 2005; Bishop et al., 2008; Holzman et al., 2008b) but also the relationships between feeding morphology, performance, and ecology (Lauder, 1983; Wainwright, 1996; Wainwright and Shaw, 1999; Collar et al., 2005; Collar and Wainwright, 2006; Wainwright, 2007; Carroll and Wainwright, 2009). This small Family contains three primary lineages (Near et al.): *Lepomis* sp. capture small evasive or attached prey with forceful suction (Collar et al., 2005; Collar and Wainwright, 2006; Holzman et al., 2012), *Micropterus* sp. capture large evasive prey using ram and high-volume suction (Higham et al.; Collar et al.; Holzman et al., 2012), and the “*Pomoxis*” clade, containing the remaining less derived and more generalized genera capture a range of prey items with relatively unspecialized suction ability (Collar et al.; Near et al.; Collar and Wainwright; Holzman et al.). Suction and ram have traditionally been considered endpoints of a

feeding performance continuum (Norton and Brainerd, 1993), and because of their differences in feeding ecology and performance, *L. macrochirus* (bluegill sunfish) and *M. salmoides* (largemouth bass) have been used as model fishes for each behavior, respectively (Carroll et al., 2004; Collar and Wainwright, 2006; Higham et al., 2006; Holzman et al., 2008a; Holzman et al., 2012). We will also use these species as representatives of feeding performance extremes, but will include *L. cyanellus* (green sunfish), a species that shares evolutionary history and morphological characteristics with *L. macrochirus* but is convergent with *M. salmoides* in capturing large evasive prey (Collar et al., 2005; Near et al., 2005; Collar and Wainwright, 2006; Collar et al., 2009) and represents a potential intermediate level of performance.

Our study has three primary objectives: 1) Model the IVW as a spheroid and generate predictive equations of IVW length and height based on data from previously published DPIV studies utilizing *L. macrochirus* and *M. salmoides*. 2) Apply this model to more natural unrestrained feeding events by quantifying 3D kinematics of *L. macrochirus*, *L. cyanellus*, and *M. salmoides* capturing evasive and non-evasive prey. 3) Determine the relationship between suction accuracy quantified under more natural conditions and capture success. Using this new technique we show that predators vary in capture success as a result of differences in the size and shape of the suction volume as well as the ability to correctly position and time the suction volume.

METHODS

Modeling the ingested volume

Original data (referred to as reference data) were from previously published DPIV studies that visualized the flow of water into the mouth of feeding *L. macrochirus* (3 individuals, 22 trials) and *M. salmoides* (3 individuals, 29 trials) (Higham et al., 2005; Higham et al., 2006). Predators were filmed in the lateral perspective capturing tethered prey items. The dimensions of the IVW were determined by manually tracking particles from mouth opening until mouth closing, and a boundary was drawn, in the frame of mouth opening, around particles that crossed the predator's jaws and were ingested (Figure 3.1, Figure 3.2 gray boundary). Further details on these methods are published elsewhere (Day et al., 2005; Higham et al., 2005; Higham et al., 2006).

From these trials, the digitized positions of the top, bottom, left, and right vertices of the boundary in the midsagittal plane of the predator (Figure 3.2, gray open circles) were used to calculate maximum IVW height (h , cm) and length (l , cm) and the ratio of height:length (H:L). The center of mass of the parcel (COP) was used to calculate the distance to the prey (d_{prey} , cm) and distance to the boundary through the prey center of mass (d_{boundary} , cm). Accuracy index (AI) was calculated using the following equation:

$$AI = 1 - \left(\frac{d_{\text{prey}}}{d_{\text{boundary}}} \right), \quad (3.1)$$

where $AI = 1$ indicated prey were located at the center of the IVW and $AI < 0$ indicated prey were located outside of the boundary. Kinematics, including ram speed (predator velocity taken at the time of peak gape, cm/s), peak gape height (greatest distance

between upper and lower jaws, cm), and time to peak gape height (TTPG; time from mouth opening to peak gape height, ms), were calculated for each trial.

To model the IVW, we simplified the shape and then predicted the dimensions using kinematics. An ellipse was used to approximate the rounded shape of the IVW in the midsagittal plane of the predator (Day et al., 2005; Higham et al., 2005; Higham et al., 2006; Day et al., 2007). Each IVW variable was then calculated using l and h as the major and minor axes of the ellipse, respectively (see supplemental methods for details) and variables were compared to the reference data using a student's t-test to validate that the elliptical IVW was similar to that of the reference IVW for each species. Following validation of the IVW shape, multiple linear regressions were used to predict IVW height (h_p) and length (l_p , dependent variables) from ram speed, peak gape height, and TTPG (independent variables), so that ellipse dimensions could be obtained without the use of DPIV. Because species were chosen to bracket the range of functional performance and prediction equations that are applicable for any species within the observed kinematic range are more useful, multiple linear regressions were carried out at the sample unit of trials ($n = 52$ trials). All possible combinations of kinematic variables were included as independent variables, and statistical models were compared using corrected Akaike Information Criteria (AICc) values (Burnham and Anderson, 2002; Anderson, 2008). Because the difference between models can be small, Akaike weights (w_i) were calculated to assess the probability that the best model was the best given the other models, and evidence ratios (ER) were calculated to determine how many times greater the best model was compared to other models (Burnham and Anderson, 2002; Anderson,

2008). Models with $ER > 3$ had little support. All calculations were performed in Matlab (R2010a, The MathWorks, Inc., Natick, MA) and code is available in the supplement. All statistics were performed in JMP (ver. 10.0, SAS Institute, Inc., Cary, NC).

Quantifying accuracy under unrestrained conditions

3D kinematics

To expand the accuracy metric to more natural behaviors, where predator and prey could respond in any direction, 3D analyses were necessary and comprise original data used to assess differences in accuracy and success among predators. Individuals were collected from Issaqueena Lake, Clemson University Experimental Forest, Clemson, SC (Table 3.1). Specimens were housed individually in 38-L aquaria maintained at 24°C, and were fed daily with commercially available frozen invertebrates (bloodworms, mosquito larvae, pieces of frozen shrimp) or live fish (wild type sailfin mollies, *Poecilia latipinna*). Fish were transferred to a 75-liter filming tank and allowed to acclimate for 1-2 days, during which time food was withheld. The Institutional Animal Care and Use Committees at Clemson University and the University of California, Riverside approved all experimental procedures.

Individuals were recorded at 500 fps (1080× 1080 pixels, Photron APX-RS, Photron USA Inc., San Diego, CA, USA) using two cameras to record lateral and ventral views (Figure 3.3) while capturing non-evasive free-floating cut pieces of shrimp or evasive live untethered mollies (filming volume 53cm x 32cm x 29cm). These prey items were chosen to elicit a range of prey capture behaviors that encompassed previously collected

Table 3.1 Species, sample sizes, and capture success rates for 3D kinematic dataset

	<i>Lepomis macrochirus</i>	<i>Lepomis cyanellus</i>	<i>Micropterus salmoides</i>
Mean SL (cm)	8.88 ± 0.91	6.57 ± 0.74	7.72 ± 2.79
Mean body mass (g)	21.11 ± 5.67	9.90 ± 4.32	10.46 ± 12.42
Capture success (%)	93.8 (non-evasive) 68.6 (evasive)	96.4 (non-evasive) 63.6 (evasive)	96.5 (non-evasive) 95.8 (evasive)
# of trials performed	134	119	105
# of individuals analyzed	5	3	5
# of trials analyzed (by individual), non-evasive	5, 5, 5, 5, 5 (<i>n</i> = 25 trials)	5, 5, 5 (<i>n</i> = 15 trials)	5, 5, 5, 5, 4 (<i>n</i> = 24 trials)
# of trials analyzed (by individual), evasive	5, 5, 6, 5, 5 (<i>n</i> = 26 trials)	4, 6, 4 (<i>n</i> = 14 trials)	5, 5, 6, 5, 5 (<i>n</i> = 26 trials)
# evasive trials analyzed that were missed capture attempts (by individual)	2, 2, 2, 0, 2	1, 3, 1	0, 0, 1, 0, 0

SL, standard length; *n*, sample size for statistical analyses on 3D kinematic data
 Values are mean ± SD

DPIV behaviors (Higham et al., 2005; Higham et al., 2006) as well as maximum performance behaviors, and both prey items were scaled to predator mouth size. Capture success was determined by scoring all trials as either a capture or miss, where misses were classified as trials in which the prey center of mass did not cross the boundary of the predator's jaws, and was determined visually after each trial. Trials in which the predator missed and then captured the prey in a subsequent attempt were scored as a miss. For each species capturing evasive prey, a combination of successful and unsuccessful trials were chosen for analysis to represent the overall capture success observed in each species (Table 3.1).

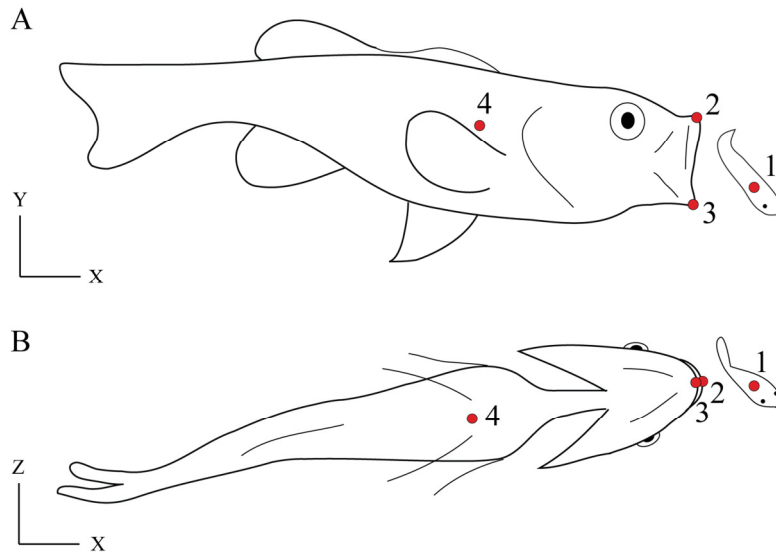


Figure 3.3 Digitized three-dimensional anatomical landmarks shown on a trace of a representative *M. salmoides* trial for the (A) lateral and (B) ventral views. 1 approximate prey center of mass, 2 tip of predator upper jaw, 3 tip of predator lower jaw, 4 approximate predator center of mass.

Trials were digitized (Figure 3.3) using DLTdv3 (Hedrick, 2008) in Matlab and all points were smoothed using a lowpass butterworth filter with a 60Hz cutoff frequency (as in Riskin et al., 2008). This value was chosen by evaluating smoothing results across a range of values for several representative videos. Smoothing was verified visually for each trial to ensure the precision of smoothed values. The midpoint of the mouth aperture was the average 3D position of both the upper and lower jaws, and was used to position the IVW relative to the predator and prey as described below (supplemental methods). The following kinematics were analyzed using the smoothed digitized points: prey velocity (mean velocity of estimated prey center of mass prior to movement induced by suction, cm/s), peak gape height (maximum distance between the upper and lower jaws, cm), time to peak gape height (TTPG; elapsed time between mouth opening and

peak gape, ms), and ram speed (change in predator center of mass position divided by the change in time between frames, taken at the time of peak gape, cm/s). Studies on centrarchids typically define mouth opening as 20%-95% of peak gape due to variable mouth opening and asymptotic gape height (Day et al., 2005; Higham et al., 2005; Higham et al., 2006), but here neither of these conditions were apparent.

Applying the IVW model to 3D dynamic capture events

Accuracy index (AI) was calculated by determining the 3D distance between the COP and the prey, and relating this to the 3D distance between the COP and the IVW boundary (through the prey center of mass) as in Equation 3.1. For details regarding modifications of the model for 3D and calculation of the boundary intersection point, see the supplemental methods. As in previous work (Higham et al., 2006), AI was also calculated in each dimension. Ingested volume (cm³) was quantified using the ellipsoid volume equation

$$\text{Ingested volume} = \frac{4\pi}{3} (l_p * w_p * h_p). \quad (3.2)$$

All kinematic analyses and calculations were performed in Matlab.

The combinations of species and prey type were chosen to represent the range of functional variation across centrarchids (suction, ram, and intermediate feeding specialization; high and low performance), and statistical analysis used these functional groups (e.g. combinations of species and prey type such as *L. macrochirus* with evasive prey) rather than nesting prey type within species. Individual variation was only significant for H:L in *M. salmoides* (Kruskal-Wallis, individuals within species as

independent variable, $\chi_4^2 = 18.39$, $p = 0.0010$) and was not significant for any other 3D IVW parameter ($p > 0.21$). Therefore, the effect of individual was considered insignificant, and trials within each functional group were used as the sampling units (Table 3.1; $n = 14$ to 26 trials for each [species x prey type] functional group).

Means were calculated for H:L, ingested volume, AI, and AI in each dimension, inclusive of misses, for each functional group. Assumptions of normality and equal variance within each functional group were tested with Shapiro-Wilks and Levene's tests respectively. Neither of these assumptions were met by all functional groups and non-parametric Kruskal-Wallis tests were performed with H:L, ingested volume, AI, or AI in each dimension as dependent variables and functional groups as independent variables. These were followed with post-hoc Dunn's multiple comparison tests to determine which groups were significantly different. Means are given with standard error except when indicated. All statistics were performed in JMP.

The relationship with capture success

To determine the relevance of predator accuracy to prey capture behaviors, a logistic regression was performed between capture success (binary dependent variable) and AI (continuous independent variable). As with the equations for predicting IVW height and length, the interest was in predictive capability across the range of prey capture behaviors among species, and logistic regression analyses were performed on evasive prey trials without accounting for species or individual ($n = 66$ trials). Logistic regression was performed using Matlab.

RESULTS

A regression-based IVW model

No significant differences in the parameters of H:L and AI were found between reference and modeled volumes, and an ellipse captured the geometry of the IVW in the midsagittal plane of the predator (Table 3.2). In the absence of known IVW vertices, height of the IVW was predicted using predator ram speed and peak gape height (Table 3.3) with the following regression equation:

$$h_p = 0.670 + (0.018 * \text{ram}) + (1.311 * \text{gape}) \quad (3.3)$$

The best regression model for predicting IVW length only included ram speed. However if ram speed is 0 cm/s (it is possible for predators to use suction with no forward movement) this would result in predicting a constant IVW length across a range of species and behaviors. Therefore, we chose to use the second best model for further analyses, which included both ram and gape (Table 3.3) with the following equation:

$$l_p = 0.927 + (0.037 * \text{ram}) + (0.611 * \text{gape}) \quad (3.4)$$

This model explained a similar amount of variation as the best model without gape (Table 3.3), and although it did result in l_p values that significantly decreased by 0.34 to 0.45 cm and significantly more circular ingested volumes, it did not result in differences in AI across species (Table 3.4). Because predators must open their mouths to generate suction and since mouth size reflects the capacity to generate suction (Carroll et al.), including gape in the regression equations essentially scaled the IVW to the predator's mouth size. Time to peak gape was not included in the best fitting model for either h_p or l_p (Table 3.3). Both regression models explained a significant portion of the variation in the

Table 3.2 Student's t-test for reference and modeled IVW parameters

Variable	Species	Reference	Modeled	<i>t</i>	<i>p</i>
H:L	<i>L. macrochirus</i>	1.15 ± 0.03	1.16 ± 0.03	-0.17	0.87
	<i>M. salmoides</i>	1.02 ± 0.03	1.00 ± 0.03	0.28	0.78
<i>d</i> _{prey}	<i>L. macrochirus</i>	0.21 ± 0.03	0.26 ± 0.03	-1.25	0.22
	<i>M. salmoides</i>	1.01 ± 0.06	0.88 ± 0.06	1.49	0.14
<i>d</i> _{boundary}	<i>L. macrochirus</i>	1.03 ± 0.04	1.00 ± 0.04	0.62	0.54
	<i>M. salmoides</i>	1.89 ± 0.07	1.93 ± 0.06	-0.44	0.66
AI	<i>L. macrochirus</i>	0.80 ± 0.02	0.74 ± 0.03	1.82	0.08
	<i>M. salmoides</i>	0.46 ± 0.03	0.54 ± 0.03	-1.84	0.07

H:L, height:length; *d*_{prey}, distance to the prey; *d*_{boundary}, distance to the boundary; AI, accuracy index
 Values for true and modeled IVW are mean ± SE.
 No variables are significantly different ($\alpha=0.05$)

Table 3.3 Multiple linear regression model selection statistics for predicting IVW height and length using the reference dataset

Predictors of IVW height	Parameters estimated	DF	SSE	RMSE	Adjusted R ²	AIC _c	<i>w</i> _{<i>i</i>}	ER
Ram + Gape	3	48	11.97	0.50	0.72	79.67*§	0.70	1.00
Ram + Gape + TTPG	4	47	11.95	0.50	0.71	82.08	0.10	6.93
Ram	2	49	13.68	0.53	0.68	84.14	0.17	4.25
Ram + TTPG	3	48	13.57	0.53	0.68	86.08	0.03	24.61
Gape	2	49	16.30	0.58	0.62	93.07	0.00	370.34
Gape + TTPG	3	48	16.14	0.58	0.62	94.91	0.00	2036.50
TTPG	2	49	41.01	0.91	0.05	140.12	0.00	6.10x10 ¹ ₂

Predictors of IVW length								
Predictors of IVW length	Parameters estimated	DF	SSE	RMSE	Adjusted R ²	AIC _c	<i>w</i> _{<i>i</i>}	ER
Ram	2	49	14.47	0.54	0.83	87.00*	0.66	1.00
Ram + Gape	3	48	14.10	0.54	0.83	88.03§	0.18	3.67
Ram + TTPG	3	48	14.34	0.55	0.83	88.88	0.12	5.62
Ram + Gape + TTPG	4	47	13.72	0.54	0.83	89.12	0.05	13.17
Gape	2	49	33.21	0.82	0.61	129.36	0.00	1.58x10 ⁹
Gape + TTPG	3	48	31.73	0.81	0.62	129.40	0.00	3.54x10 ⁹
TTPG	2	49	83.41	1.30	0.02	176.33	0.00	2.50x10 ¹ ₉

Predictors are ordered by AIC_c value.
 TTPG, time to peak gape height; DF, degrees of freedom; SSE, error sum of squares; RMSE, root mean squared error; AIC_c, corrected Akaike information criterion; *w*_{*i*}, Akaike weight; ER, evidence ratio
 * Best choice model (smallest AIC_c)
 § Model used in further analyses

Table 3.4 Repeated measures t-test results comparing predicted length equations with and without gape included

Variable	Statistic	Bluegill sunfish (DF = 50)	Green sunfish (DF = 28)	Largemouth bass (DF = 49)
predicted length	Difference in means	-0.338	-0.413	-0.4504
	t	-17.155	-13.446	-11.911
	p	<0.0001*	<0.0001*	<0.0001*
H:L	Difference in means	0.091	0.718	0.074
	t	13.876	24.119	10.969
	p	<0.0001*	<0.0001*	<0.0001*
d_{prey}	Difference in means	-0.0294	-0.0158	-0.0431
	t	-3.621	-0.808	-5.149
	p	0.0007*	0.4258	<0.0001*
d_{boundary}	Difference in means	-0.0882	-0.989	-0.1537
	t	-7.458	-4.092	-7.776
	p	<0.0001*	0.0003*	<0.0001*
AI	Difference in means	-0.0045	-0.0107	0.0034
	t	-1.328	-1.625	1.186
	p	0.1902	0.1154	0.2412

Difference in means is the mean value when gape is included minus the mean value when gape is not included.
* Significantly different at $p=0.05$ (also marked yellow)

reference values of each parameter (h_p : ANOVA, $F_{2,48} = 64.04$, $p < 0.0001$; l_p : ANOVA, $F_{2,48} = 123.31$, $p < 0.0001$), and the predicted values were similar to the actual values calculated using the reference dataset (Figure 3.4).

Applying suction accuracy to more natural 3D trials

Success was high for all species when capturing non-evasive prey, but varied when capturing evasive prey (Table 3.1). Whereas *M. salmoides* had similarly high success rates with both prey types, both *L. macrochirus* and *L. cyanellus* decreased success rates by 25.2% and 32.8% respectively when capturing evasive prey. Differences across prey

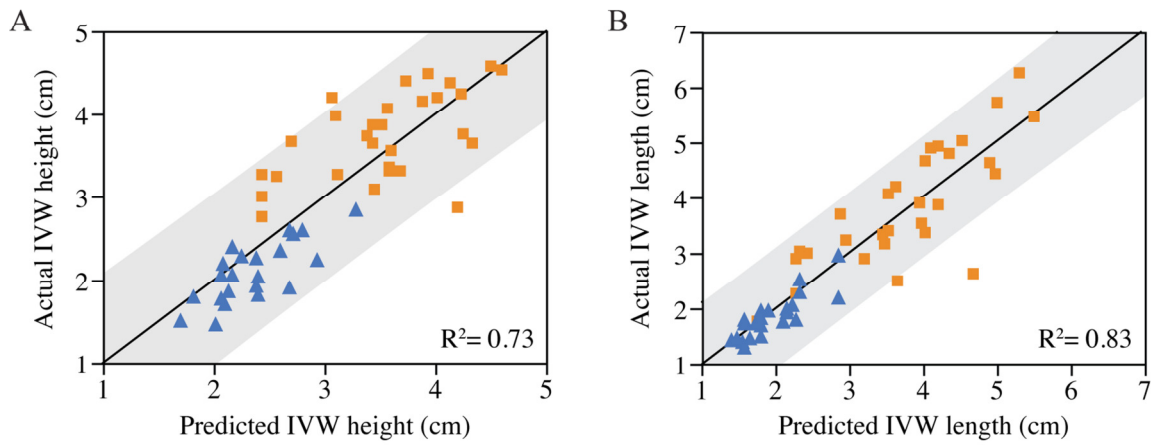


Figure 3.4 The relationship between predicted and actual values (from the reference dataset) of IVW (a) height and (b) length. Height and length were predicted using multiple linear regressions with ram speed and gape height as predictors (Table 3.3). *L. macrochirus* trials are shown with blue triangles and *M. salmoides* trials are shown with orange squares. The regression line and 95% prediction intervals are also shown with a black line and gray shading, respectively. These intervals indicate the ability to obtain each actual value at a given predicted value.

types were also apparent with kinematics (Table 3.5). Ram speeds ranged from 22.1 ± 2.4 cm/s in *L. macrochirus* capturing non-evasive prey to 114.3 ± 4.9 cm/s in *M. salmoides* capturing evasive prey and peak gape height ranged from 0.83 ± 0.05 cm to 1.21 ± 0.07 cm in the same species and prey types. Therefore, these functional groups represent observed extremes in kinematic performance in this study. Ram speed and peak gape height in *L. cyanellus* were intermediate to the other species when capturing evasive prey, but were both greater than the other species for non-evasive prey, indicating that performance differences between prey types was not as dramatic in *L. cyanellus*.

The IVW was modeled in three dimensions (Figure 3.5) and kinematic differences led to significant differences in the shape (H:L; Kruskal-Wallis, $\chi^2_5 = 63.34, p <$

0.0001) and size (ingested volume; Kruskal-Wallis, $\chi^2_5 = 76.90, p < 0.0001$) of the IVW (Table 3.5, Figure 3.6). The ratio of H:L ranged from 0.74 ± 0.015 (elongate) for *M. salmoides* capturing evasive prey to 0.96 ± 0.015 (nearly circular) for *L. macrochirus* capturing non-evasive prey, supporting the idea that these two functional groups represent extremes in performance. Additionally, the H:L of *L. macrochirus* capturing non-evasive prey was significantly greater than any species capturing evasive prey, and *L. cyanellus* was the only species where H:L was not different across prey types (Figure 3.6). Differences in size of the ingested volume mirrored those observed for H:L. The greatest ingested volume was observed for *M. salmoides* capturing evasive prey and was greater than all other functional groups with the exception of *L. cyanellus* capturing evasive prey. The smallest volume predicted, that of *L. macrochirus* capturing non-evasive prey, was only 21.1% of the size of the largest volume. Again, *L. cyanellus* was the only species for which there were no significant differences in ingested volume across prey types. These differences in IVW shape and size are apparent when the mean IVW is visualized (Figure 3.7).

Accuracy index was generally high across functional groups with a maximum of 0.79 ± 0.02 in *L. macrochirus* capturing non-evasive prey. However, although there were significant differences (Kruskal-Wallis, $\chi^2_5 = 19.83, p = 0.0013$), these were few (Figure 3.5). Specifically, *L. macrochirus* capturing evasive prey had the poorest accuracy, and this was significantly lower than both *L. macrochirus* capturing non-evasive prey and *M. salmoides* capturing evasive prey (Dunn's multiple comparison tests, $z > 3.07, p < 0.03$). For both piscivores, although accuracy index was reduced with

Table 3.5 Mean 3D kinematics and modeled IVW parameters

Variable	<i>L. macrochirus</i>		<i>L. cyanellus</i>		<i>M. salmoides</i>	
	NE	E	NE	E	NE	E
Ram (cm/s)	22.08 ± 2.4	68.00 ± 5.3	65.13 ± 9.2	89.61 ± 6.6	47.81 ± 7.4	114.29 ± 4.9
Gape (cm)	0.83 ± 0.05	1.05 ± 0.03	1.00 ± 0.04	1.08 ± 0.05	0.90 ± 0.07	1.21 ± 0.07
TTPG (ms)	53.8 ± 3.6	28.3 ± 1.2	31.9 ± 2.8	28.9 ± 2.5	30.5 ± 2.5	25.1 ± 2.1
h_p (cm)	2.15 ± 0.09	3.28 ± 0.11	3.16 ± 0.20	3.69 ± 0.17	2.71 ± 0.19	4.31 ± 0.11
l_p (cm)	2.25 ± 0.11	4.09 ± 0.20	3.95 ± 0.35	4.90 ± 0.27	3.25 ± 0.30	5.89 ± 0.17
H:L	0.96 ± 0.015	0.82 ± 0.014	0.83 ± 0.025	0.76 ± 0.009	0.86 ± 0.020	0.74 ± 0.015
d_{prey} (cm)	0.23 ± 0.023	0.90 ± 0.125	0.48 ± 0.077	0.90 ± 0.202	0.43 ± 0.061	0.62 ± 0.061
$d_{boundary}$ (cm)	1.10 ± 0.05	1.85 ± 0.10	1.83 ± 0.15	2.15 ± 0.14	1.55 ± 0.15	2.62 ± 0.08
AI	0.79 ± 0.02	0.51 ± 0.07	0.74 ± 0.03	0.59 ± 0.10	0.73 ± 0.02	0.77 ± 0.02
AI _x	0.88 ± 0.02	0.69 ± 0.05	0.81 ± 0.04	0.72 ± 0.09	0.80 ± 0.02	0.83 ± 0.03
AI _y	0.90 ± 0.02	0.76 ± 0.05	0.91 ± 0.02	0.86 ± 0.03	0.90 ± 0.01	0.91 ± 0.01
AI _z	0.91 ± 0.02	0.80 ± 0.05	0.94 ± 0.02	0.79 ± 0.06	0.91 ± 0.02	0.95 ± 0.01
Ingested volume (cm ³)	6.3 ± 0.9	25.6 ± 3.2	25.2 ± 5.5	38.5 ± 6.1	18.2 ± 4.8	59.9 ± 4.4

NE, non-evasive prey; E, evasive prey; TTPG, time to peak gape height; h_p , predicted height; l_p , predicted length, H:L, height:length ratio; AI, accuracy index; AI_x, accuracy along the X-axis; AI_y, accuracy along the Y-axis; AI_z, accuracy along the Z-axis
 Values are mean ± SE
 Significant differences among means are discussed in the text.

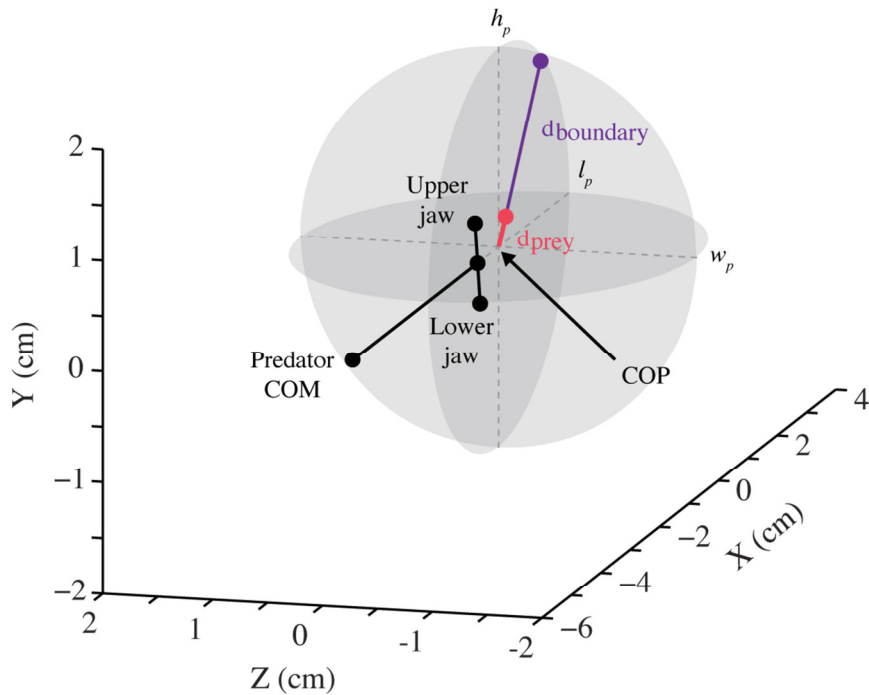


Figure 3.5 Three dimensional reconstruction of a representative *L. macrochirus* trial showing the positions of predator (large black dots) and prey (red dot) landmarks at 4 ms prior to prey capture. The estimated ingested volume of water (IVW) is overlain using grey shaded ellipses representing X, Y, and Z dimensions. Predicted height (h_p), length (l_p), and width (w_p) of the volume are shown with dashed gray lines. Accuracy is calculated following Figure 3.2, where d_p (red line) is the 3D distance to the prey and d_b (purple line) is the 3D distance to the spheroid boundary through the prey center of mass. Parameters for this trial are as follows: ram 86.5 cm/s, gape 1.06 cm, time to peak gape 32 ms, mean prey velocity 13.5 cm/s, l_p 4.78 cm, h_p and w_p 3.62 cm, H:L 0.76, d_p 0.30 cm, d_b 1.81 cm, AI 0.83, ingested volume 32.68 cm³.

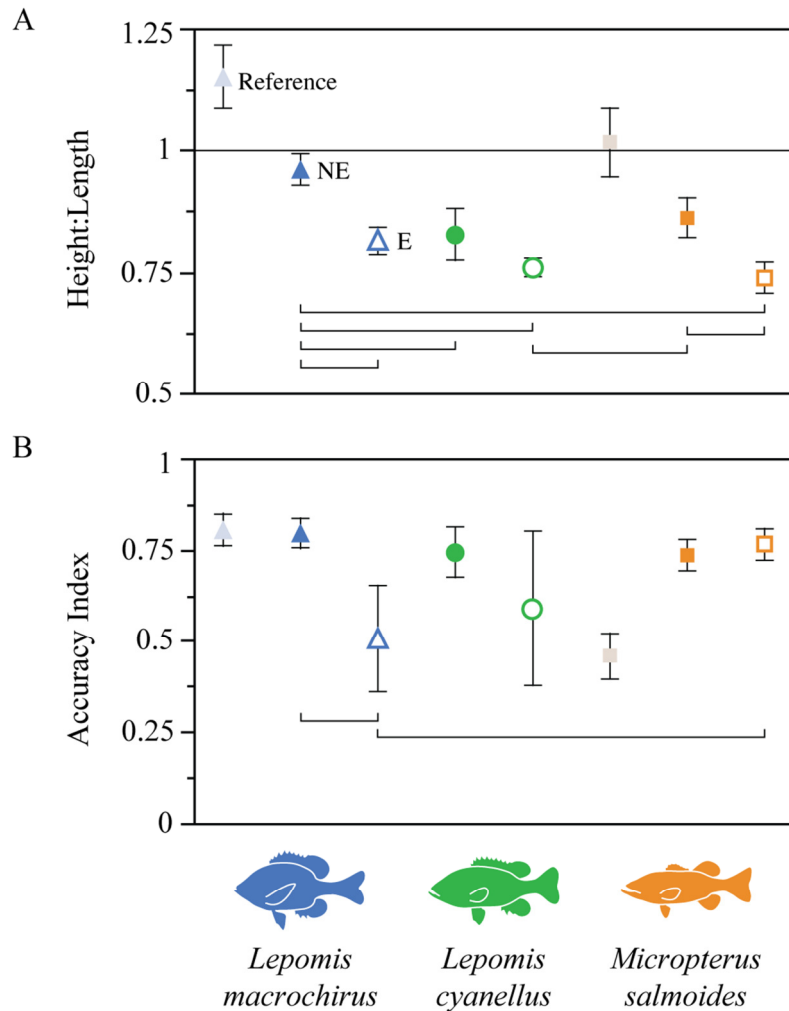


Figure 3.6 Species means and 95% confidence intervals for (A) H:L ratio and (B) accuracy index. Comparisons are shown for 2D reference data obtained with particle image velocimetry techniques (Higham et al., 2005; Higham et al., 2006) for *Lepomis macrochirus* and *Micropterus salmoides* (shown for comparison, light filled shapes), as well as for 3D modeled estimates for all three species capturing non-evasive (NE; unfilled shapes) and evasive (E; dark filled shapes) prey types. A line is drawn at H:L = 1 to indicate the value at which the ingested volume of water is circular. A color-coded figure demonstrating general body shape is also shown for each species. Significant differences are indicated by the brackets, where species that are linked are significantly different (Dunn's multiple comparison tests, $p < 0.02$).

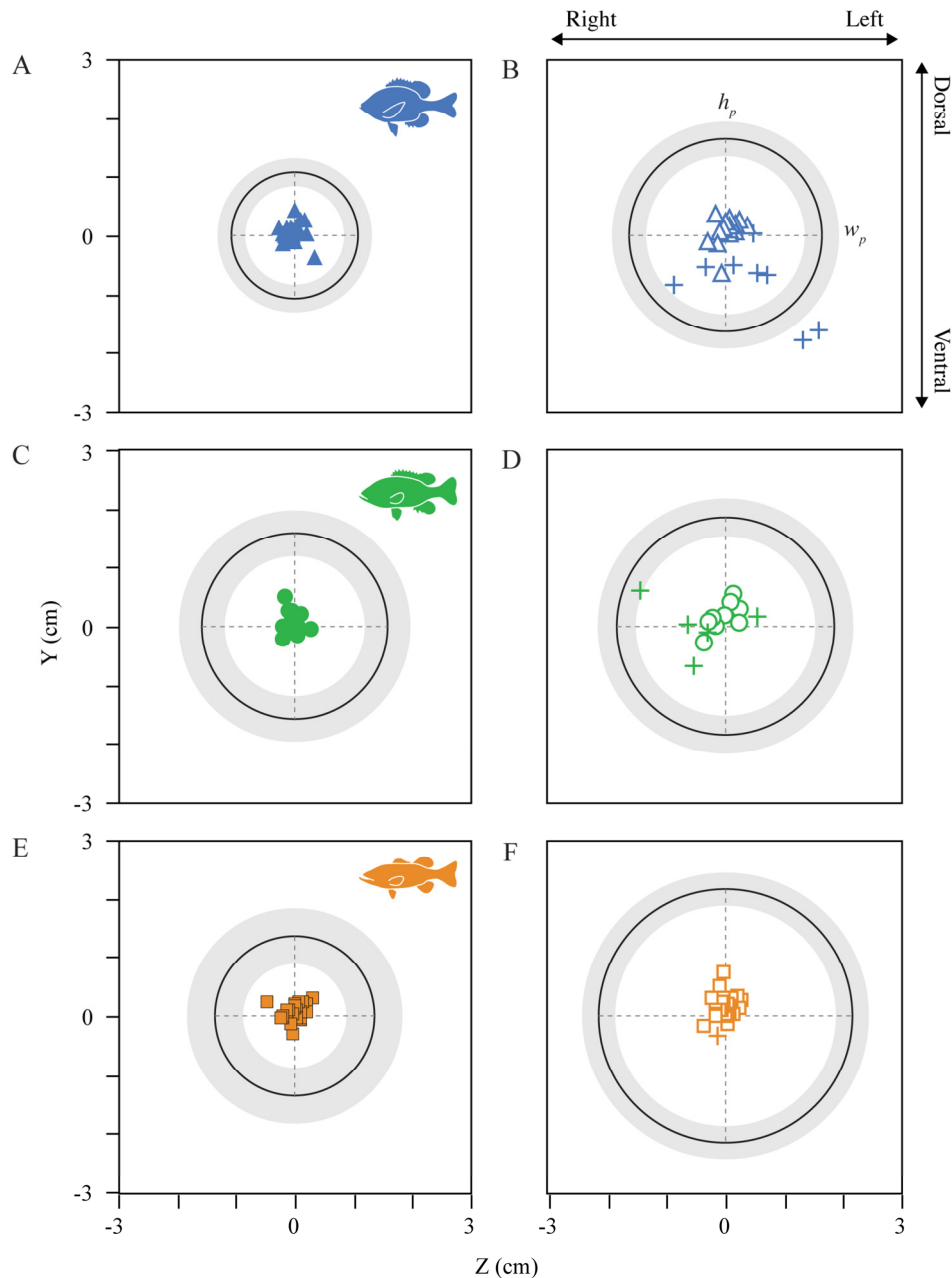


Figure 3.7 Position of prey relative to the modeled IVW in the frontal view of the predator (as though the predator is swimming toward the viewer), shown for (A, B) *L. macrochirus*, (C, D) *L. cyanellus*, and (E, F) *M. salmoides* capturing non-evasive (A, C, E) and evasive (B, D, F) prey types. Markers follow Figure 3.6 with the addition of missed prey capture attempts shown as + symbols. Prey positions are plotted for each trial, with an ellipse that represents the mean \pm SD (black line and gray shading, respectively) IVW shape for each functional group. Predicted height (h_p) and width (w_p) axes are also shown as dashed lines.

evasive prey, this difference was not significant (Dunn's multiple comparison tests, *L. cyanellus*, $z = 1.362$, $p = 1.00$, *M. salmoides*, $z = -0.896$, $p = 1.00$). In examining AI in each dimension, significant differences were only observed in the X- (Kruskal-Wallis, $\chi_5^2 = 12.45$, $p = 0.0291$) and Z-dimensions (Kruskal-Wallis, $\chi_5^2 = 20.72$, $p = 0.0009$). Specifically, AI_x was greater for *L. macrochirus* capturing non-evasive prey than for the same species capturing evasive prey (Dunn's multiple comparison tests, $z = 3.30$, $p = 0.0146$). Additionally, AI_z was greater for *M. salmoides* capturing evasive prey than either other species with the same prey (Dunn's multiple comparison tests, *L. macrochirus*, $z = 3.33$, $p = 0.013$, *L. cyanellus*, $z = 3.70$, $p = 0.0032$). For all functional groups, accuracy was poorest in the X-dimension, along the path of forward trajectory (Table 3.5), non-evasive prey were located closer to the COP than evasive prey, and prey that were missed were positioned farther from the COP than successful captures (Figure 3.7).

Accuracy and capture success

Accuracy index explained 52.7% of capture success. The estimated probability of success (\hat{p}) can be predicted using the following equation:

$$\ln\left(\frac{\hat{p}}{1 - \hat{p}}\right) = -5.059 + (11.038 * AI) \quad (3.5)$$

The odds ratio was calculated by scaling the regression coefficient for AI by the standard deviation (SD) for AI (SD = 0.30). Predators were 30 times more likely to successfully

capture prey with a SD increase in AI. Strikes where AI was less than 0 (indicating prey were located outside the modeled IVW boundary) always resulted in missed capture attempts (Figure 3.8).

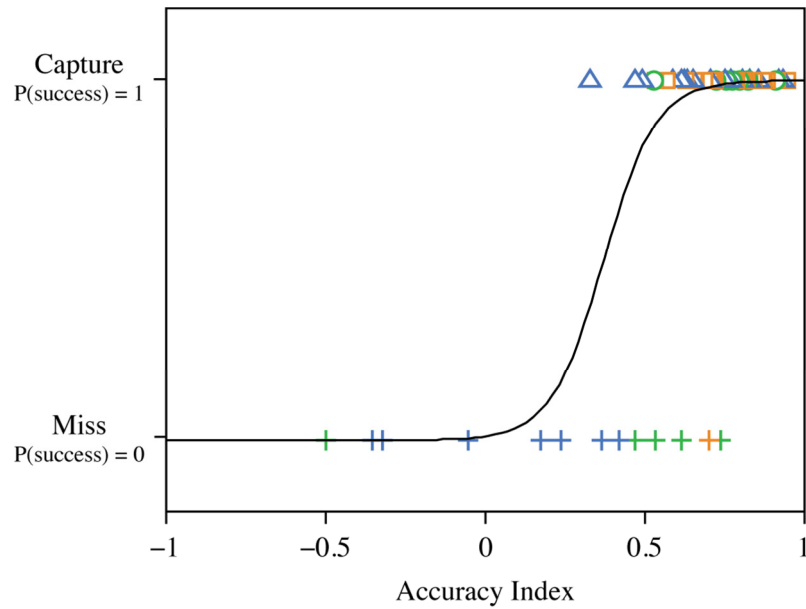


Figure 3.8 Logistic regression of the probability of capture success for 3D modeled trials. Accuracy explains 52.7% of capture success. Marker style follows Figure 3.7.

DISCUSSION

We present a novel method to determine predator accuracy in suction-feeding fishes by predicting the shape of the IVW and relating prey position to the center of this volume in three dimensions. Unlike prior methods (Higham et al., 2006; Nauwelaerts et al., 2008), our model can be used when predator and prey are unconstrained, which more closely matches natural predator-prey interactions. Additionally, this model only requires kinematics, and is therefore more efficient for analyzing a large number of individuals and/or species. We applied this model to more natural prey capture events for three species of closely related predators capturing evasive and non-evasive prey and show that accuracy predicts success. Our model makes IVW parameters and suction accuracy more accessible measures of prey capture performance that can be used to understand predator strategies and generate large-scale hypotheses of prey capture performance evolution.

Model performance and limitations

Our model of the IVW was used to quantify suction accuracy under more natural conditions, and significantly explained > 50% of the variation in capture success. Therefore, our model is a reasonable estimate of the ability for predators to correctly position and time their strike relative to the prey (Drost, 1987). However, in comparison to previously published work using DPIV analyses, differences in accuracy between species were not replicated. Whereas *L. macrochirus* (AI = 0.80) was 74% more accurate than *M. salmoides* (AI = 0.46) (Higham et al., 2006), in this study the trend was reversed, particularly with evasive prey: *M. salmoides* (AI = 0.77) was 51% more accurate than *L.*

macrochirus (AI = 0.51). This indicates that the experimental techniques used in each study may present bias toward the feeding behaviors of each species. Since *L. macrochirus* is specialized for capturing attached prey with forceful suction (Collar et al., 2005; Collar and Wainwright, 2006; Holzman et al., 2012), presenting predators with tethered prey could artificially inflate the performance of *L. macrochirus* relative to *M. salmoides*. Alternatively, presenting predators with free-swimming evasive fishes, as in the current study, could favor the strategy of *M. salmoides*. Therefore, future studies should include multiple prey types to accurately account for specialization among species. Specifically, we suggest the three prey types defined in Holzman (Holzman et al., 2012): large evasive, small evasive, and attached prey. Each of these prey types require divergent functional demands and these demands have been demonstrated as axes of diversification among centrarchids (Collar and Wainwright, 2006; Collar et al., 2009; Holzman et al., 2012).

Our model of the IVW and estimates of accuracy predict capture success and support the use of accuracy as a performance measure (Irschick et al., 2008), and it is a necessary link for understanding suction performance. Some of the earliest models of suction behaviors were useful for describing the detailed hydrodynamics of suction feeding (Weihs, 1980; Muller et al., 1982; Muller and Osse, 1984; van Leeuwen and Muller, 1984), but none of these models address whether the event results in successful capture. Two approaches have recently been developed to understand differences in capture success across suction-feeding predators: the Suction-Induced Flow Field (SIFF) model (Holzman et al., 2012), and accuracy relative to the IVW (Higham et al., 2006;

Nauwelaerts et al., 2008). The SIFF model uses kinematics, fluid flow, and prey characteristics to predict hydrodynamic forces experienced by prey as a measure of a predator's ability to entrain a prey item (Holzman et al., 2012). Whereas SIFF simplifies predator-prey interactions and predicts what species should be capable of, the IVW model relies on trial-level kinematics to understand what actually happens during a feeding event. Therefore, these two models are complimentary – SIFF predicts capture success and the IVW model explains capture success with accuracy. By expanding on the DPIV technique to capture a wider range of predatory behaviors, our IVW-based model of suction performance represents a significant step toward linking feeding performance with feeding success, and can be used to better understand why suction-feeding predators are more or less successful on divergent prey types.

All models are simplifications of natural phenomena, and as such, impose limitations on their interpretations and applicability. Our IVW model has three primary assumptions: 1) that modifications of the IVW are the primary means of modulating accuracy, 2) that an ellipsoid (or more technically, a spheroid) approximates the ingested volume, and 3) that prey positioned closer to the COP represents greater accuracy. Predators can control the position of the IVW relative to the prey by protruding their jaws at an angle, thereby deflecting the IVW in the respective direction (as in sharks Nauwelaerts et al., 2008), by altering their orientation during the approach to the prey (personal observation), or by modifying the hydrodynamics to affect the reach and volume of suction (Higham et al., 2006). Since the species we used have terminal mouths, we assume deflection was negligible. We also assume that by the time of the

strike the predator has completed its orientation behaviors to facilitate the use of lateral musculature for powering mouth expansion (Carroll et al., 2004; Camp and Brainerd, In press). Our work supports the second assumption that an ellipse approximates the shape of the ingested volume in the midsagittal plane of the predator based on 2D, constrained prey capture events, but this may not be the case if mouth shape is not circular at all points of mouth opening (as indicated in Van Leeuwen, 1984) or if predator direction varies during the strike (e.g. pitch during braking). However, it is difficult to assess 3D hydrodynamics of suction in moving predators at this time, and this simplified model provides reasonable estimates that can be fine-tuned given further developments. The final assumption regarding the position within the volume representing the greatest accuracy has never been addressed, but we provide data indicating that it may not be entirely correct.

Studies that have quantified suction accuracy using the IVW have assumed that the greatest accuracy occurs when prey are located at the COP (Higham et al., 2006; Nauwelaerts et al., 2008). However this has never been verified by statistically testing whether position along any axis affects success. It can be argued that for prey items that are located at 25% and 75% of the length of the X axis, projected forward of the predator along the centerline (Figure 3.3), AI would be 0.5 in both cases but the prey at 25% would be encountered prior to peak gape whereas the prey at 75% would be encountered after, giving the predator a greater chance of capturing the prey at 25% compared to the one at 75%. Using our data on capture success, we calculated the position of the prey as a percentage along the X, Y, and Z axes and performed logistic regressions to determine

if success was biased by prey position along any axis. We found that predators have an increased chance of success when prey are located closer to the predator on the X axis and higher in the volume on the Y axis. There was no effect of position along the Z axis (predator's left vs. right) on predator success. Therefore, had our measure of accuracy accounted for these biases toward success, we may have found a stronger relationship between accuracy and success. However, since we were able to establish a relationship between accuracy and success, the effect of this bias may only be slight. This represents a significant caveat to our model, and future work should address a method for accounting for positional biases when quantifying accuracy.

This study was performed on adult fishes where viscous forces are negligible during prey capture and suction flow is unidirectional, and therefore our model has limited applications for small or larval fishes where viscous forces could have a significant effect on suction performance (Reynold's number < 200) (Fuiman and Webb, 1988; Hernandez, 2000) or secondarily aquatic vertebrates where suction flow is bidirectional (Lauder and Shaffer, 1986; Miller and Larsen Jr, 1989). Note also that as predator size reaches its lower extreme in larval fishes, mouth size and ram speed may approach 0 and the predicted length and height of the IVW might converge toward 0.967 and 0.670, but this relationship between IVW length and height at small predator sizes has not been verified. However, our model can be applied to the majority of cases and is therefore appropriate as a starting point. Centrarchids were chosen in this study due to the extensive body of research supporting hydrodynamic and kinematic relationships, but additional taxa should also be assessed and tested against DPIV analyses to verify the applicability of our model

to other species. The utility of the IVW model comes from the ability to assess performance across a range of suction-feeding predators and to generate macroevolutionary hypotheses of prey capture evolution.

Factors that affect suction accuracy

Holzman *et al.* outline three primary factors determining the outcome of a suction-feeding predation attempt: the predator's strategy for approaching prey, the predator's ability to perform feeding behaviors, and the ability of the prey to respond to the strike (Holzman *et al.*, pg. 8). We argue that suction accuracy is an emergent property of all three factors and is an integrative measure of capture performance. Suction accuracy will decrease if the predator startles the prey during the approach, if the predator fails to generate peak forces at the correct time and position relative to the prey, and if the prey responds correctly away from the predator. Therefore, to maintain accuracy and ensure success, predators must integrate approach and feeding behaviors to prevent or overcome prey responses. Although prey behavior plays a large role in predator success (Walker *et al.*, 2005; Holzman *et al.*, 2012; Stewart *et al.*, 2013) and preliminary data support the role of prey behavior in determining predator success (unpublished data), we were not able to address prey behavior directly so we focus our discussion on the role of predator behavior.

Predators often modulate kinematics in response to prey type to increase the chance of success on evasive prey (Nemeth, 1997a) and these changes are likely the mechanism for modulating the size and shape of the IVW. Predators attack evasive prey faster and

from a greater distance (Nyberg, 1971; Norton, 1991; Norton and Brainerd, 1993; Nemeth, 1997a; Wainwright et al., 2001) presumably to reduce the amount of time for prey to escape. Additionally, predators use a greater magnitude and rate of cranial expansion (Ferry-Graham et al., 2001; Van Wassenbergh and De Rechter, 2011) leading to an increased magnitude of buccal pressure change (Norton and Brainerd, 1993; Nemeth, 1997b) and therefore suction. Interestingly, although reduced time to peak gape (rate of expansion) increases peak fluid speed, and therefore suction force (Day et al., 2005; Higham et al., 2005), it is not a strong predictor of suction volume (Higham et al., 2006). This may be due to the interaction between timing and magnitude, where faster expansion results in less time to ingest particles, limiting any substantial influence on volume change.

We found that predators increased ram speed and gape size when capturing evasive prey, resulting in larger and more elongate ingested volumes in two of three predators (Table 3.4, Figures 3.6). This modulation presumably increased the chance of predator success on evasive prey, but in *L. macrochirus* suction accuracy was low and this modulation was not extensive enough to achieve the level of success observed in *M. salmoides*. This may be due to a small mouth size that limits the ability to capture larger prey. Alternatively, the large gape and high ram speed of *M. salmoides* is effective at capturing non-evasive prey at reduced magnitudes. Modulation was not observed in *L. cyanellus*, resulting in poor success. This species was not allowed to ambush prey from a hiding spot (as was common in holding tanks), which may be a more optimal strategy to prevent detection by prey and could explain similar IVW characteristics regardless of

prey type. However, in general, the relationships between kinematics and suction volume provide the mechanism for changing the shape and size of the IVW when predators encounter evasive and non-evasive prey types, which represents a novel level of performance modulation.

Suction accuracy refers to the ability to correctly position and time the suction volume relative to the prey, and predators can control this accuracy in two ways: by modifying body orientation and position or by modifying the size and shape of the IVW. These two behaviors are not mutually exclusive since forward movement increases the volume of particles ingested by suction (Higham et al., 2005; Higham et al., 2006). We show how the size and shape of the IVW is modified with changes in peak gape height and ram speed at peak gape, and these variables represent the roles of feeding and locomotor performance in prey capture. Therefore, the ability to coordinate feeding and locomotor behaviors may be a key factor maximizing success. Surprisingly most differences between species and prey types were in shape and size of the ingested volume, rather than suction accuracy. Similar levels of accuracy observed across species may be achieved by compensating for feeding performance with approach strategies, or vice versa. Typically, feeding and locomotion are considered independent performance regimes, but during prey capture the ability to integrate these behaviors in a way that results in timing and positioning the IVW correctly with respect to the prey may be critical for ensuring success.

Ecological relevance

Several studies have attempted to describe the ability of suction-feeding predators to correctly position and time their strikes on prey (Drost, 1987; Higham et al., 2006; Nauwelaerts et al., 2008; Holzman et al., 2012), but none have empirically tested the hypothesis that suction accuracy predicts success. Our work supports this hypothesis, and by establishing the link between suction accuracy and success we provide the ecological relevance of accuracy for suction-feeding predators (Irschick et al., 2008). Accuracy requires integration of locomotor and feeding behaviors so that the suction volume is correctly positioned and timed relative to the prey, and in this way it represents a more holistic measure of performance, and adds insight into the prey capture strategies of predators.

Our study only examined the differences in three species of centrarchid predators, but the accessibility of suction accuracy as a measure of performance makes it possible to examine differences across a wider range of taxa. The next steps should be to determine whether the patterns observed in these species are also reflected in more generalized species such as those in the “*Pomoxis*” clade, or mulluscivorous species specialized for prey processing, rather than capturing (e.g. *L. microlophus* or *L. gibbosus*). Additionally, patterns of accuracy and capture success should be examined in other non-Centrarchid taxa to validate the generality of our study. Our work suggests mechanisms by which ecological and evolutionary specialization affect the ability to capture prey, but to address this question empirically, macroevolutionary analyses of prey capture performance and success are necessary.

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SUPPLEMENTAL METHODS

Modeling the ingested volume

To model the ingested volume as a spheroid (where two of the ellipsoid axes are the same length), we began with the digitized vertices from previous work and used these to calculate IVW length and height to be used as the major and minor axes of an ellipse. Top, bottom, left, and right vertices (given from previous work) were rotated to the predator's frame of reference. Two dimensional distances between left and right vertices (l) and top and bottom vertices (h) were used as the major and minor axes of the ellipse, respectively following the Pythagorean theorem for the hypotenuse of a triangle. Note that this differs from the original calculation of l and h where the radii were half of the 1-dimensional distance along each axis (for example, h was the difference in Y-values for the top and bottom points, divided by 2). The center of the ellipse at the intersection of l and h represented the modeled center of the parcel of water (COP). Again this differs from the original calculation where the COP was the mean X and Y position for all four points. The 2D distance from the modeled COP to the prey (d_{prey}) was then calculated. To determine the distance to the ellipse boundary through the prey center of mass (d_{boundary}), the coordinates of the intersection of a line from the COP to the ellipse boundary, through the prey, with the ellipse were calculated as follows:

$$x = \left(\left(\frac{l}{2} \right) * \cos \theta \right) \quad (\text{S3.1})$$

$$y = \left(\left(\frac{h}{2} \right) * \sin \theta \right) \quad (\text{S3.2})$$

where θ is the parametric angle of the prey relative to the X-axis. The 2D distance between this point and the COP (d_{boundary}), IVW height:length ratio (H:L) and accuracy index (AI) were then calculated for all trials and compared to the values obtained previously with reference PIV data. Predicting l and h allowed the vertices to be positioned along X and Y axes using the center of the predator's mouth as the proximal vertex and approximating the position of maximum height at 50% of IVW length (Higham et al., 2005; Higham et al., 2006). Although maximum height occurred at slightly greater than 50% of maximum length for the reference IVW dataset (Figure 3.1; test $H_0: \mu = 50\%$; *L. macrochirus*: mean \pm SD = $52.4 \pm 5.48\%$, $t_{21} = 2.09$, $p = 0.0491$; *M. salmoides*: mean \pm SD = $55.6 \pm 4.44\%$, $t_{28} = 6.77$, $p < 0.0001$), to simplify the model this was not accounted for.

2.2 Quantifying accuracy under more natural conditions

2.2.2 Applying the IVW model to 3D dynamic capture events

Modifications were necessary to apply the IVW model to 3D predator-prey interactions. First, the shape of the volume was estimated as an ellipsoid, or more technically a spheroid, rather than an ellipse. Due to symmetrical flows expected from the circular shape of the mouth aperture at the time of prey capture (Day et al., 2005; Skorczewski et al., 2012), the predicted width of the volume (w_p) was equal to h_p . However, visualizing this volume and determining prey position relative to the center of the volume must account for both prey and predator movement. Because prey were free

to move or escape during the feeding event, prey could be located near or outside the IVW boundary at the time of mouth opening but could potentially be moving in a direction facilitating their capture. For this reason, we used the position just prior to suction-induced prey movement (4 ms prior to prey center of mass crossing the predator's jaws, as determined from representative videos) as this represents the final moment for prey to control its movement relative to the predator and the suction volume. We did not use the time of peak gape, when suction forces and accuracy are greatest (Day et al., 2005; Higham et al., 2006), because prey were often located within the mouth cavity at this time. However, for an unsuccessful strike, where time of prey capture was unobtainable, position of predator and prey were taken at the time of peak gape. Additionally, because the predator moves toward the prey during the strike, the predator is located within the IVW at 4 ms prior to capture, so the IVW cannot be projected entirely forward of the predator's mouth as is shown in Figure 3.2. Instead, since the length axis represents time (see Introduction), distance traveled by the predator as a proportion of distance traveled between opening and peak gape was used to scale the position of the predator along the length axis of the IVW so that the predator was located within the IVW. For example, if a predator traveled $2/3$ of the distance between opening and peak gape, only $1/3$ of the radius ($l_p/2$) was projected in front of the predator's mouth. Prey location was maintained relative to the predator, so scaling the position of the predator in this way allowed for proper placement of the IVW relative to the prey. This calculation determined the position of the COP, and all ellipse vertices were determined by adding or subtracting radii lengths along each axis.

Once these modifications were made, the intersection with the spheroid boundary was calculated. To simplify calculation of the position of vertices and to standardize trials to the same orientation, the 3D orientation of the predator was used to rotate the entire system of points into the predator's frame of reference (i.e. rotation was 0° about each of the X, Y, and Z axes). We calculated the intersection of the line from the COP through the prey center of mass to the spheroid boundary (d_{boundary} , Figure 3.3) using the equations for a 3D ellipsoid

$$\left(\frac{x}{a}\right)^2 + \left(\frac{y}{b}\right)^2 + \left(\frac{z}{c}\right)^2 = 1 \quad (\text{S3.3})$$

where (x, y, z) are the coordinates of the origin and a , b , and c are the radii along the corresponding dimension, and the 3D line

$$(u, v, w) = (\lambda * (x_1, y_1, z_1)) + ((1 - \lambda) * (x_2, y_2, z_2)) \quad (\text{S3.4})$$

where (u, v, w) are the coordinates for the point of interest, and (x_1, y_1, z_1) and (x_2, y_2, z_2) are the coordinates for the known points on the line (COP and prey location, respectively). The constant λ ranges from 0 and 1 and is solved by substituting equation S3.4 into equation S3.3,

$$\begin{aligned} & \left(\frac{(\lambda * x_1) + ((1 - \lambda) * x_2)}{a}\right)^2 + \left(\frac{(\lambda * y_1) + ((1 - \lambda) * y_2)}{b}\right)^2 \\ & + \left(\frac{(\lambda * z_1) + ((1 - \lambda) * z_2)}{c}\right)^2 = 1 \end{aligned} \quad (\text{S3.5})$$

Since the COP was located at (0,0,0), equation 3.4 can be simplified to

$$\left(\frac{(1-\lambda) * x_2}{a}\right)^2 + \left(\frac{(1-\lambda) * y_2}{b}\right)^2 + \left(\frac{(1-\lambda) * z_2}{c}\right)^2 = 1 \quad (\text{S3.6})$$

and is further reduced so that

$$\lambda = 1 - \sqrt{\frac{1}{\left(\frac{x_2}{a}\right)^2 + \left(\frac{y_2}{b}\right)^2 + \left(\frac{z_2}{c}\right)^2}} \quad (\text{S3.7})$$

Having solved for λ and knowing the coordinates for the COP and prey, the point of intersection of the line from the COP to the boundary, through the prey, was then calculated following equation S3.3, and d_{boundary} and AI were calculated. These calculations were performed for each trial.

CHAPTER 4:

The Causes and Consequences of Integration

INTRODUCTION

Integration refers to the ability for parts of an organism to work together in a coordinated way (Bayliss, 1921; Olson and Miller, 1958; Gould and Lewontin, 1979; Zweers, 1979; Seaborg, 1999; Pigliucci, 2003), and is measured as the statistical association among traits, where strong correlation indicates strong integration (Olson and Miller, 1951; Olson and Miller, 1958; Gould and Lewontin, 1979; Cheverud, 1982; Wainwright et al., 2008). Much of the work quantifying organism-level integration has centered on morphological traits, and the interest has been in the mechanism and variability of integration, as well as the evolutionary implications (Peres-Neto and Magnan, 2004; Badyaev et al., 2005; Marugán-Lobón and Buscalioni, 2006; Collar et al., 2008; Mitteroecker and Bookstein, 2008; Porto et al., 2009; Klingenberg et al., 2011). This extensive effort has led to methodological advances in quantifying integration as a multivariate phenomena within and across taxa (Rohlf and Corti, 2000; Adams and Felice, 2014), and has demonstrated that integration among morphological traits is readily apparent (reviewed in Klingenberg, 2008). This work has been important for establishing the role of morphological integration in describing differences among organisms and evolutionary trajectories. However, if selection acts on behavior and morphological changes are indirect (Garland Jr, 1994; Garland Jr. and Losos, 1994), then performance integration, or the ability for organisms to integrate behaviors in a

meaningful way during ecologically relevant tasks, may be more relevant for understanding the factors governing organism survival and evolution (Irschick et al., 2008). However, since measuring performance of a single trait can often be time consuming and difficult, few have attempted to understand multiple interacting levels of performance, and little is known about the mechanism and variability of performance integration.

Performance integration was suggested as an area of research on its own by Higham only recently (Higham et al., 2005; Higham et al., 2006; Higham, 2007b; Higham, 2007a) and although several approaches to measuring integration have been used to examine these ideas (Rice and Westneat, 2005; Higham, 2007a; McElroy et al., 2008; Rice, 2008; Montuelle et al., 2009; Montuelle et al., 2012b; Oufiero et al., 2012; Bimbard et al., 2013), none have been able to directly explain integration of multivariate performance datasets. This may partially be due to unfamiliarity with a statistical technique widely used in studies of morphological integration (Klingenberg and Zaklan, 2000; Rohlf and Corti, 2000; Zelditch et al., 2004) as well as other diverse research areas (Wold et al., 2001; Boulesteix and Strimmer, 2007; Carrascal et al., 2009; Qureshi and Compeau, 2009; Krishnan et al., 2011) that does exactly this. Partial least squares (PLS) is an ordination technique that extracts singular axes (SA) to describe the latent patterns underlying the covariation between two multivariate datasets (Wold, 1975; de Jong, 1993; Garthwaite, 1994; Wold et al., 2001; Haenlein and Kaplan, 2004; Abdi, 2010). Following ordination, axes can then be correlated or regressed to determine the statistical association between datasets (Zelditch et al., 2004; Abdi, 2010; McGuire, 2010). Other

ordination techniques, such as principal components analysis (PCA) have also been used to reduce data into axes that can be regressed or correlated (Figure 4.1) (Collar et al., 2008; Montuelle et al., 2009; Montuelle et al., 2012a). However, this method indirectly describes the relationship between datasets because PCA axes describe the variation within each dataset separately, and if the axes of variation in each dataset are not important in defining the relationships between datasets, then correlations between PCA axes may not be an accurate descriptor of integration between systems (Mevik and Wehrens, 2007). Therefore, because PLS is designed to maximize covariance, correlations between PLS axes more directly quantify integration and PLS is a more appropriate test for understanding the statistical association between performance datasets. Another advantage of PLS is that it is robust to both Type I and Type II error and can be used when there are fewer samples than the number of variables, collinearity among variables is high, and predictor and response matrices are multivariate (Mevik and Wehrens, 2007; Carrascal et al., 2009). These characteristics are important in performance studies when samples may be difficult to obtain and traits are often correlated. Therefore, PLS correlations provide a useful statistical test for assessing performance integration between multiple performance variables simultaneously. This study will use PLS to analyze locomotor and feeding performance during prey capture in fishes as a model system for understanding the nature of integration between performance traits. These systems are useful because performance has been studied extensively in separate contexts (Harris, 1936; Liem, 1978; Lauder, 1982; Webb, 1982b; Motta, 1984; Wainwright, 1996; Domenici and Blake, 1997; Wainwright and Shaw,

1999; Westneat et al., 1999; Lauder, 2000b; Wainwright et al., 2002; Walker and Westneat, 2002; Blake, 2004; Dean et al., 2007; Wainwright et al., 2007) but the reliance on locomotion during prey capture is expected (Webb, 1984b; Wainwright et al., 2001; Higham, 2007b) and has been supported with univariate analyses (Higham, 2007b; Higham, 2007a; Kane and Higham, 2011; Oufiero et al., 2012). Locomotor and feeding systems are highly modular (although axial muscles are involved in mouth opening (Camp and Brainerd, 2014)) and can be used separately with success. Because of this, their combined use suggests that integration is the result of functional constraints on prey capture performance rather than shared developmental or neural pathways (e.g. in taxa that use the same structure for both behaviors (Altenbach, 1989; Nekaris and Rasmussen, 2003)). Additionally, performance traits across these systems are not likely additive, but rather comprise a tradeoff that must be mitigated during integrated behaviors. During feeding, performance is greatest when water flow (suction) generated by the mouth is fastest (Day et al., 2005; Day et al., 2007); during forward movement, performance is greatest when the body position is translated rapidly (Domenici and Blake, 1997). However, during prey capture a tradeoff exists so that species with stronger suction approach at lower velocity and *vice versa* (Higham et al., 2006). Therefore, integration represents an emergent level of performance that is not apparent by studying each system in isolation (Kane and Higham, 2011), and can provide important insights into constraints on organismal performance. Additionally, quantifying integration in a multivariate context will allow for a better understanding of how multiple variables interact, which is an insight that is not possible with univariate analyses (as in Kane and Higham, 2011).

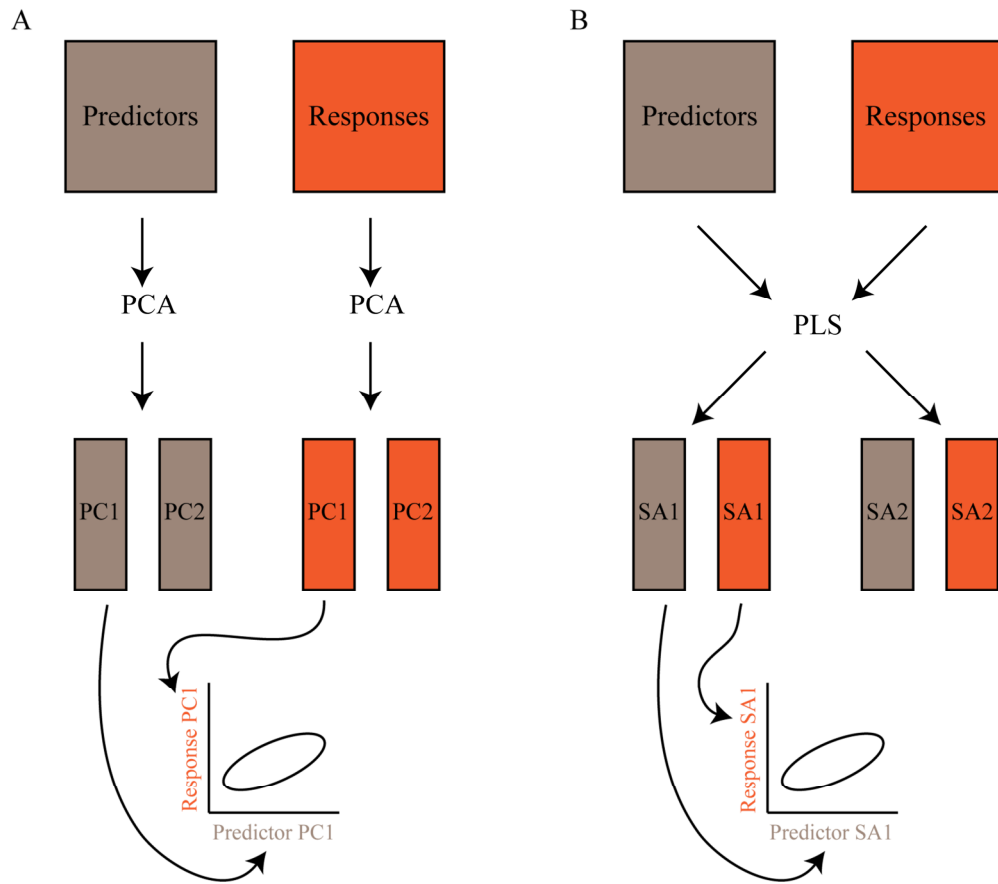


Figure 4.1 Differences between A) principal components analysis (PCA) correlation and B) partial least squares (PLS) correlation. Both ordination techniques summarize predictor and response datasets and use axes for each dataset to determine correlations (shown graphically with an ellipse). However, PLS summarizes the variation in each dataset separately, whereas PLS summarizes the covariation between datasets.

Fishes are also an ideal system because studies of locomotion and feeding have suggested factors that might affect a species' ability to be integrated, but none of these hypotheses have been tested. For example, attack strategy may be important for defining integration, since faster predators have been shown to increase coordination of feeding muscle activity and kinematics (Liem, 1978; Oufiero et al., 2012). Additionally the

correlation between peak gape and ram speed at peak gape was stronger in a high speed predator (Higham, 2007a). Specialization for locomotor or feeding behaviors may also affect integration due to a greater need for behaviors to work together, or as a factor of constrained options available (Webb, 1984b; Higham, 2007b; Kane and Higham, 2011). Predators have been shown to modulate feeding kinematics and swimming speed in response to prey type (Anderson, 1993; Lemell and Weisgram, 1996; Nemeth, 1997; Ferry-Graham et al., 2001; Matott et al., 2005; Van Wassenbergh et al., 2006; Van Wassenbergh and De Rechter, 2011; Kane and Higham, 2014) but whether this modulation results in changes in integration is unknown. Finally, peak gape and ram at peak gape (Higham, 2007a; Higham et al., 2007; Oufiero et al., 2012; Kane and Higham, 2014), or morphological traits directly related to these abilities (Higham, 2007b; Collar et al., 2008) have been the primary variables used to represent locomotion and feeding, but recent work suggests that these may not be the only integrated variables (Kane and Higham, 2011). Predator accuracy is important for successful prey capture (Kane and Higham, 2014) and is estimated with measures of velocity and gape. Therefore, the ability to integrate locomotor and feeding behaviors may be important for correctly positioning and timing the predator relative to prey and ensuring success. For this reason, understanding the mechanisms and variability of integration are important first steps toward understanding how organisms perform and survive in their environment.

This study will examine locomotor and feeding performance during evasive and non-evasive prey capture events in *L. macrochirus* to begin to understand integrated performance traits in a multivariate context. This analysis will use bluegill (*Lepomis*

macrochirus) trials collected in previous work (Kane and Higham, 2014). This subset was chosen because variation in accuracy and success was high in this species, and large differences in kinematics between prey types were observed. Therefore, of the three species, bluegill are the most likely to exhibit variation in integration. The aim of the current work is to determine whether multivariate integration exists, and if so, whether integration can be modulated between prey types, whether modulation results in changes in locomotor and feeding kinematics involved in integration, and whether integration has consequences for predator accuracy. Additionally, because PLS has not been used in this context, performance of PLS will be assessed by manipulating the variables included in analysis and comparing the results to PCA and univariate correlations. These analyses will not only demonstrate the usefulness of a new statistical technique, but will inform our understanding of what drives performance integration in organisms and why this might be important in an ecological context.

METHODS

Quantifying kinematics

Evasive and non-evasive prey trials from *Lepomis macrochirus* (bluegill sunfish; 9.27 ± 0.56 cm standard length, 23.91 ± 3.19g) used in Chapter 3 were also used here. For further details on housing and filming, see (Kane and Higham, 2014). All experimental procedures were approved by the Institutional Animal Care and Use Committees at Clemson University and University of California, Riverside.

Accuracy (Kane and Higham, 2014) was quantified using kinematic landmarks that only described movements of the jaws and predator center of mass during the attack (mouth opening to mouth closing). Here, these data were expanded to include additional locomotor movements during the approach and post-attack, and additional feeding movements (e.g. cranial rotation, jaw protrusion, gular displacement, and opercular expansion). In total, 10 points were digitized (Hedrick, 2008) from lateral and ventral

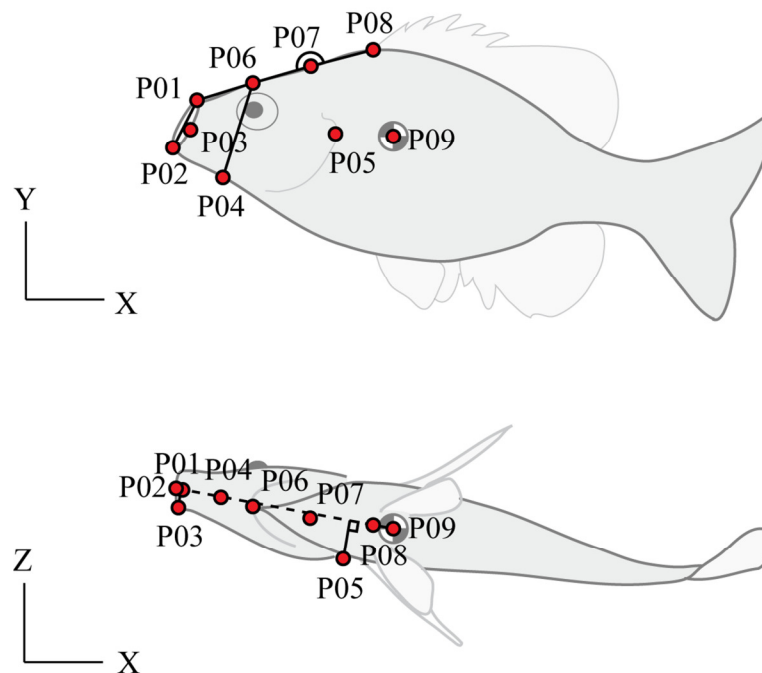


Figure 4.2 Digitized points in lateral and ventral view shown at the time of peak gape. Points are as follows: P01 upper jaw, P02 lower jaw, P03 tip of the descending process of the premaxilla, P04 floor of the mouth (estimated visually), P05 opercular lobe, P06 center of the head above the eye, P07 vertex of cranial rotation (estimated visually), P08 dorsal fin insertion, P09 estimated predator center of mass. Distance and angle calculations are shown with black lines and are described in the text. An additional point was digitized in a single frame (at the beginning of the sequence when the predator's body was the most straightened and the mouth had not yet opened) at the tip of the ventral lobe of the tail, and was used with P01 to calculate video-estimated predator total length.

perspectives (Figure 4.2). The start of the sequence began at the first sign of forward movement by the predator and ended at maximum pectoral fin abduction during braking after the feeding event. This resulted in sequences approximately 700 ms in duration. To minimize digitizing error, all digitized points were smoothed using a quantic spline (R2010a, The MathWorks, Inc., Natick, MA). Tolerance values were set manually and visually inspected for each point before proceeding to further calculations. This was done to ensure the integrity of smoothed values.

Performance refers to what happens as a result of the parts moving (kinematics), and for locomotion this means body movement through space, whereas for feeding this means suction generation. Locomotor performance can therefore be quantified using measures of center of mass translation through time (Figures 4.3A and 4.4A). However, suction can only be directly quantified by visualizing water movement, which is a difficult and invasive technique. Instead, kinematics provide a direct link between mouth expansion and suction force (Sanford and Wainwright, 2002; Day et al., 2005), and for this reason, kinematics describing excursions of the jaws, gular region, operculum, and cranium through time (Figures 4.3B and 4.4B) will be used as a proxy of suction performance. The locomotor dataset (LOCO) consisted of the following measurements describing predator movement: velocity (cm/s) and acceleration (cm/s^2) at the time of peak gape, mean approach velocity (cm/s; prior to mouth opening), peak velocity (cm/s), peak acceleration (cm/s^2), peak deceleration (cm/s^2), and time of peak velocity, acceleration,

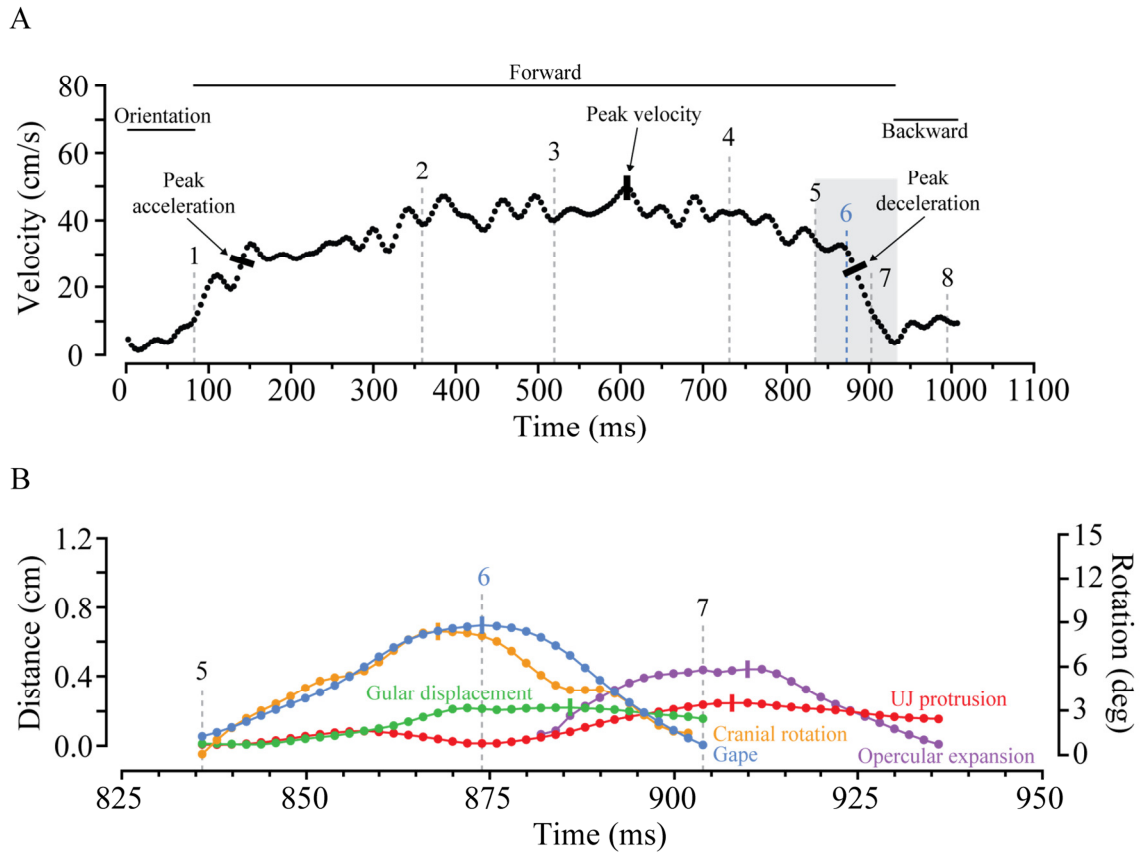


Figure 4.3 Representative non-evasive prey kinematics of predator A) center of mass and B) cranial movements through time. For clarity, datapoints in A were downsampled to 250 frames/s although kinematics were calculated relative to the complete 500 frames/s dataset. Peak magnitudes are shown with small lines within each trace, and markers signifying peak gape are colored blue to match panel B. The gray shaded box in A represents the time period corresponding to B. Kinematic events are shown with numbered light gray vertical dashed lines and are as follows: 1 start of the forward approach (84 ms), 2 protraction of pectoral fins during approach (360 ms), 3 glide during approach (520 ms), 4 glide during approach (732 ms), 5 mouth opening (836 ms), 6 peak gape (874 ms), 7 mouth closing and peak dorsal and anal fin excursion (904 ms), 8 peak pectoral fin protraction and end of the capture event (996 ms).

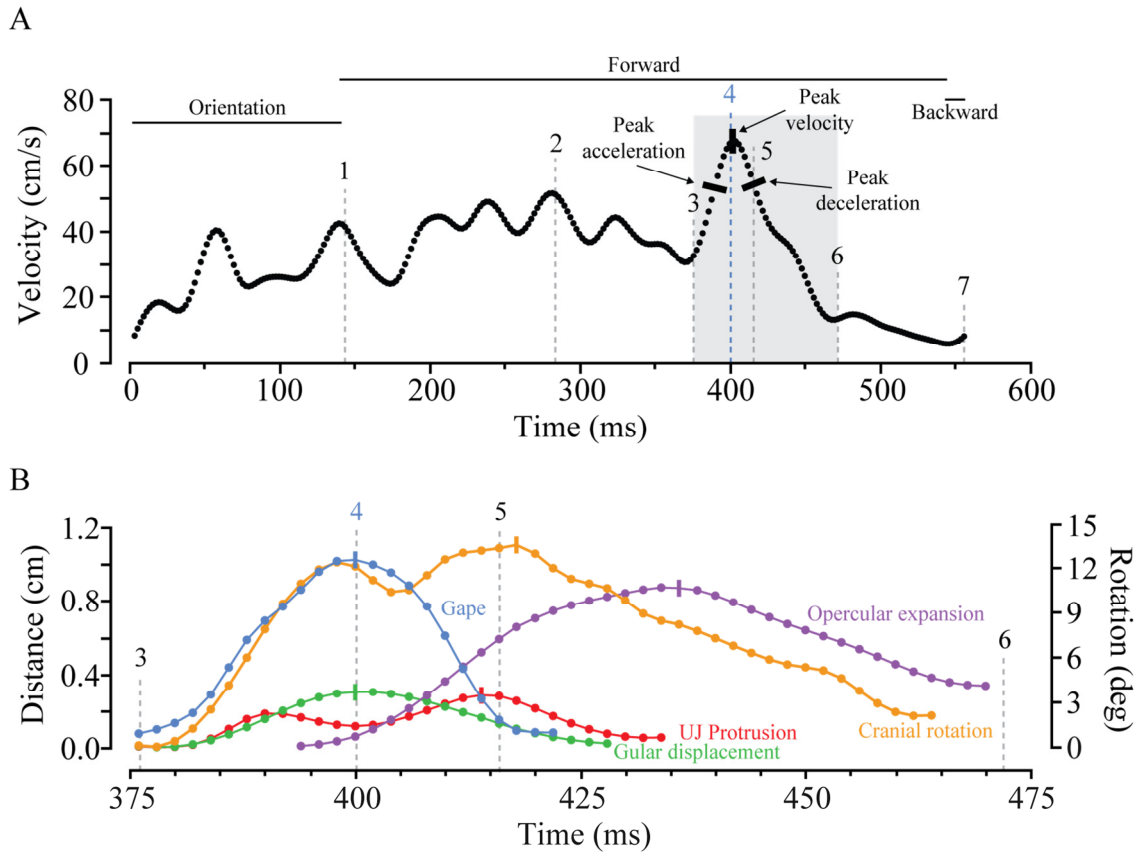


Figure 4.4 Representative evasive prey kinematics of predator A) center of mass and B) cranial movements through time. Markers follow figure 4. Kinematic events are as follows: 1 start of the forward approach (144 ms), 2 glide during approach (284 ms), 3 mouth opening and start of prey escape response (376 ms), 4 peak gape (400 ms), 5 mouth closing (416 ms), peak dorsal and anal fin excursion (472 ms), peak pectoral fin protraction and end of the capture event (556 ms).

and deceleration (ms; standardized relative to the time of peak gape). The feeding dataset (FEED) consisted of the following measurements describing predator buccal cavity expansion (shown in Figure 4.2): peak gape (cm; distance between P01 and P02), time to peak gape (ms; duration from mouth opening to peak gape), peak mouth aperture area (cm²; area of the ellipse formed using gape height and the perpendicular distance from P03 to gape height), peak upper jaw protrusion (cm; distance between P06 and P01) and protrusion at peak gape, peak gular displacement (cm; distance between P06 and P04), peak opercular expansion (cm; perpendicular distance between P05 and the midline, drawn from P09 to the midpoint of the line describing gape), peak cranial rotation (deg; angle formed by P06, P07, and P08) and rotation at peak gape, and time to all peaks (ms; standardized relative to the time of peak gape). All kinematic calculations and measurements were performed in Matlab.

Statistical analyses

To account for slight differences in body size among individuals, all linear distances were divided by predator total length as measured from one frame in each video (3D distance from upper jaw to lower lobe of tail). These measures were preferred over measures of standard length taken from euthanized specimens because, despite careful calibration, predators that are closer to the camera could be perceived as being larger. Within each variable, normality was tested using Shapiro Wilk's tests and the assumption of equal variance was tested using Levene's test. Differences in means between prey

types were compared using either t-tests or non-parametric Kruskal-Wallis tests (if assumptions were not met in one or more groups).

A partial least squares (PLS) ordination was used to determine the combination of LOCO and FEED variables that best explain the covariation between these datasets (Wold, 1975; de Jong, 1993; Garthwaite, 1994; Wold et al., 2001; Haenlein and Kaplan, 2004; Abdi, 2010). In PLS, predictor and response matrices are used as input and each resulting SA is composed of pairs of scores that relate to either predictors or responses. A plot of SA1 response vs. SA1 predictors can then be used to visualize the relationship between matrices (Figure 4.1). For this study, LOCO was used as the predictor and FEED was used as the response. To assess differences in integration between prey types, both prey types were included in the PLS and subsequent analyses assessed variation in PLS scores due to prey type. This technique is also used in information systems studies to assess between-group differences using PLS (Qureshi and Compeau, 2009). However, a PLS was also performed within each prey type to confirm that the combined analysis captured the patterns observed within each prey type. The number of significant singular axes for each PLS was determined using the “leave-one-out” method of cross-validation.

To determine the magnitude of integration and the contribution of locomotor and feeding variables to observed levels of integration, several tests were performed on the SA scores for each dataset. Correlations between PLS axes (FEED SA1 vs. LOCO SA1) across and within prey types were used to test the statistical association (integration) of locomotor and feeding performance variables. Correlations were preferred over regressions because they are symmetric and there is no expectation for one dataset to

predict the other. However, for visualization purposes, FEED-related data are shown on the *y*-axis and LOCO-related data are shown on the *x*-axis because most locomotor behaviors occur prior to mouth opening, reducing the ability for feeding to predict locomotion. Steiger's *Z* test was used to determine whether correlations were significantly different between groups (Steiger, 1980). In addition to differences in integration (correlation), a student's *t*-test was used to test for differences in means for each prey type along each PLS axis, to determine whether bluegill use different kinematic patterns to capture each prey type. To test the sensitivity of PLS to kinematic variable inputs, PLS was re-run excluding the four (two upper and lower) variables with the most extreme loadings for each axis. These variables have the strongest influence on the relationship between datasets because of their strong loadings, and excluding these variables should have the strongest potential influence on PLS results. Differences between correlation coefficients before and after variable exclusion were tested using Steiger's *Z* test.

To facilitate comparisons with other methods that have been used to quantify integration, two additional methods of quantifying integration were also performed. Principal component analysis (PCA) and factor analysis have also been used to summarize datasets, and correlation or regression relationships between components have been used as an alternative means of determining integration (Collar et al., 2008; Montuelle et al., 2009; Montuelle et al., 2012a). Therefore, PCA correlations were also performed on the data derived in this chapter to compare the results with PLS correlation. PCA was run for each dataset (LOCO and FEED), and only the first principal

components (PCs) were correlated against each other. As with the PLS correlations, these were determined for each prey type from a combined analysis and the same variable exclusion tests were also run for PCA axes. A second quantification method used univariate correlations among all LOCO and FEED variables. This is similar to early work on performance integration (Higham et al., 2006; Higham, 2007b; Higham et al., 2007; Kane and Higham, 2011).

Finally, an exploratory analysis of the relationship between accuracy (Kane and Higham, 2014) and integration was performed to assess the ecological relevance of integration for predatory fishes. However, because integration was represented as a correlation coefficient across many trials and accuracy was represented as a value for each trial, regression residuals for each trial were instead used to represent deviation from integration (the line of fit). Major axis (or orthogonal) regression accounts for deviation in both the X and Y dimensions simultaneously by minimizing the sum of the squared perpendicular distances (Sokal and Rohlf, 1995). Because deviation from integration can occur in both dimensions, major axis regression was performed on PLS ordination scores (combined analysis, all variables) and the perpendicular distances were saved as residuals. Accuracy was then regressed with these perpendicular residuals to determine if integration predicts predator accuracy (or more technically, if deviation from integration predicts poor accuracy). All statistical analyses were performed in JMP 11 (SAS Institute, Inc., Cary, NC) except Steiger's Z tests, which were performed using a web-based calculator (<http://vassarstats.net/rdiff.html>).

RESULTS

Predators approached prey by first orienting to the prey, then swimming directly toward the prey primarily using movements of the pectoral and caudal fins. The caudal fin was used to accelerate toward prey at the time of mouth opening when capturing evasive prey. Buccal expansion was initiated by opening the jaws and depressing the floor of the mouth. Cranial rotation and upper jaw protrusion either occurred near-simultaneously with peak gape, or showed an initial submaximal peak prior to peak gape, followed by a maximal peak after the time of peak gape. Buccal expansion terminated posteriorly with opercular expansion, which occurred during and after mouth closing. Deceleration was initiated during feeding, initially by protraction of the dorsal and anal fins, followed by protraction of the pectoral fins and caudal fin. Pelvic fins were generally protracted throughout the duration.

Predators modulated 10 of 23 measured kinematic parameters when capturing evasive compared to non-evasive prey types (Table 4.1). Time to peak gape was faster and the magnitudes of peak gape, gular displacement, and opercular expansion were greater when capturing evasive prey. Additionally, all velocity measures were faster, and the timing of peak velocity, acceleration, and deceleration were closer to the time of peak gape when capturing evasive prey. Therefore, non-evasive prey generated low-performance feeding and locomotor behaviors whereas evasive prey evoked high-performance behaviors. Non-evasive prey were captured over a longer duration and from a greater distance compared to evasive prey.

Table 4.1 Mean (\pm SE) kinematics for each prey type

Variables	Non-evasive (n=15 trials)	Evasive (n=16 trials)	Test statistic (<i>t</i> or <i>z</i>)	<i>p</i>
<i>LOCO</i>				
Velocity at PG (BL/s) ¹	2.2 \pm 0.4	5.6 \pm 0.3	-4.210	<0.0001*
Acceleration at PG (BL/s ²) ^{1,2}	-35.3 \pm 8.5	-121.1 \pm 50.3	1.443	0.1491
Mean approach velocity (BL/s) ¹	2.6 \pm 0.3	3.3 \pm 0.2	-2.352	0.0187*
Peak velocity (BL/s) ¹	4.3 \pm 0.4	6.8 \pm 0.5	-3.498	0.0005*
Time of peak velocity (ms relative to PG) ¹	-319.07 \pm 37.34	-48.88 \pm 24.96	-4.174	<0.0001*
Peak acceleration (BL/s ²) ^{1,2}	204.4 \pm 27.4	360.9 \pm 66.9	-1.719	0.0855
Time of peak acceleration (ms relative to PG) ¹	-456.00 \pm 50.67	-82.88 \pm 26.08	-4.231	<0.0001*
Peak deceleration (BL/s ²) ^{1,2}	-202.5 \pm 25.9	-350.3 \pm 67.4	1.878	0.0604
Time of peak deceleration (ms relative to PG) ¹	-288.40 \pm 46.45	-93.50 \pm 45.81	-2.848	0.0044*
<i>FEED</i>				
Peak gape (cm) ¹	0.083 \pm 0.005	0.097 \pm 0.003	-2.154	0.0312*
Time to peak gape (ms) ^{1,2}	45.87 \pm 3.89	27.75 \pm 0.91	4.023	<0.0001*
Peak aperture area (cm ²)	20.12 \pm 2.22	23.71 \pm 1.41	-1.383	0.1771
Time of peak aperture area (ms relative to PG) ¹	0.27 \pm 0.18	0.38 \pm 0.20	-0.372	0.7098
Upper jaw protrusion at PG (BL)	0.016 \pm 0.003	0.014 \pm 0.003	0.477	0.637
Peak upper jaw protrusion (BL)	0.024 \pm 0.002	0.026 \pm 0.002	-0.714	0.4807
Time of peak upper jaw protrusion (ms relative to PG)	13.47 \pm 3.75	13.88 \pm 3.66	-0.078	0.9384
Peak gular displacement (BL)	0.023 \pm 0.002	0.034 \pm 0.003	-3.130	0.004*
Time of peak gular displacement (ms relative to PG) ¹	3.60 \pm 2.17	2.63 \pm 0.83	0.060	0.952
Peak opercular expansion (BL) ¹	0.046 \pm 0.004	0.063 \pm 0.005	-2.629	0.0086*
Time of peak opercular expansion (ms relative to PG)	30.53 \pm 1.92	34.75 \pm 1.68	-1.659	-0.1079
Cranial rotation at PG (deg)	9.50 \pm 1.13	9.95 \pm 0.97	-0.299	0.7674
Peak cranial rotation (deg)	11.86 \pm 0.87	12.34 \pm 0.66	-0.441	0.6622
Time of peak cranial rotation (ms relative to PG) ¹	-5.87 \pm 4.10	4.63 \pm 3.10	-1.569	0.1166

¹ Variable was not normally distributed for one or more prey types

² Assumption of equal variances was not met

In cases where assumptions were not met, a non-parametric Kruskal-Wallis test was used; otherwise, a t-test was used to test for differences between groups.

Distance measures are given in units of body length. Mean total length as measured from videos was 11.82 \pm 0.145 cm.

Partial least squares correlation

A partial least squares (PLS) ordination was used to determine the combination of variables that describe the covariation between locomotion and feeding kinematics, and the correlation between axes was used to indicate integration between datasets. The LOCO dataset was significantly and positively correlated with the FEED dataset ($r = 0.83, p < 0.0001$) indicating that certain combinations of kinematics are observed together, and that changes in locomotor kinematics result in correlated changes in feeding kinematics (Figure 4.5A). Only one singular axis (SA) was significant, and whereas locomotor kinematics explained 52% of the variation in FEED, feeding kinematics only explained 15% of the variation in LOCO. The LOCO axis was primarily defined by weak locomotor performance (magnitudes) that occur early in the feeding event in the negative direction and strong performance as well as timing closer to the time of peak gape in the positive direction (Table 4.2, Figure 4.5B). The FEED axis was primarily defined by weak performance (slower mouth opening and lesser magnitude of expansion) in the negative direction and strong performance (faster mouth opening and greater magnitude of expansion) in the positive direction (Table 4.2, Figure 4.5B).

To determine differences in integration between prey types, correlations between PLS scores were determined within each prey type. Each prey type occupied distinct regions of the ordination space (Figure 4.5A) indicating that predators rely on different combinations of feeding and locomotor kinematics for capturing alternative prey types. Specifically, evasive prey trials were located at more positive values along each axis than non-evasive prey trials (t-test, LOCO: $t_{29} = -6.60, p < 0.0001$; welch's test, FEED:

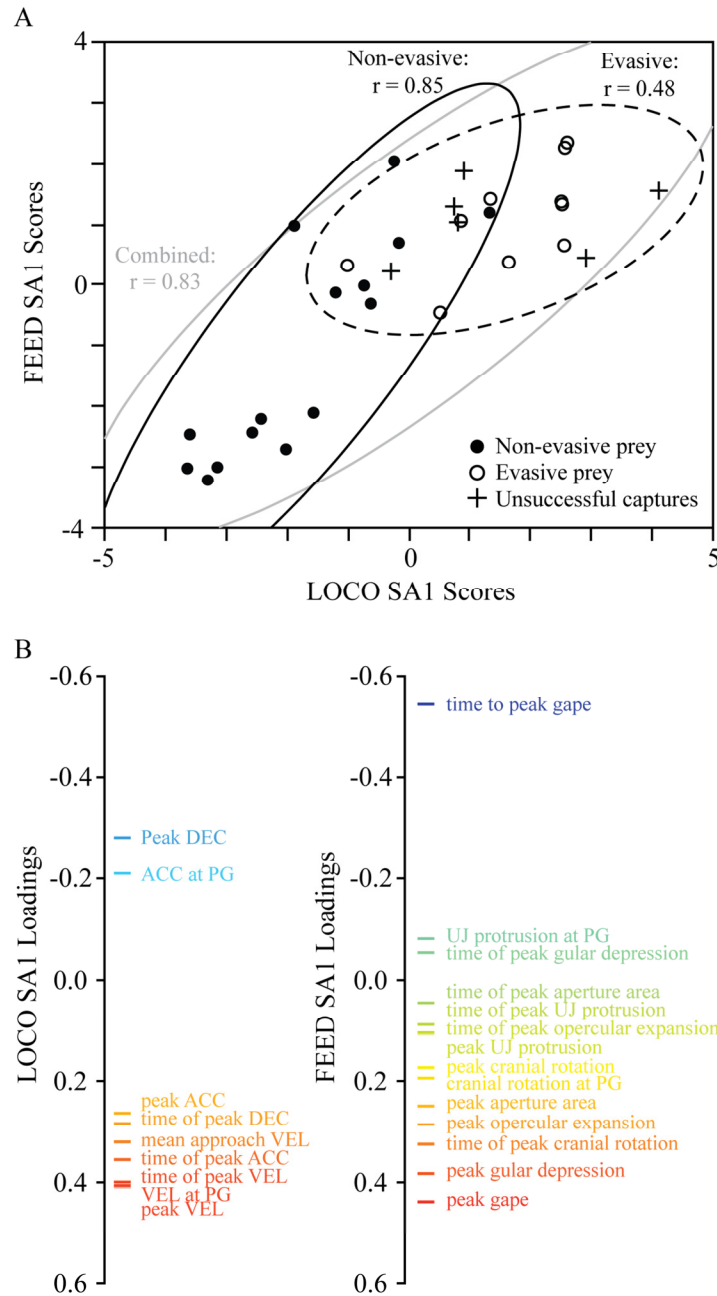


Figure 4.5 Partial least squares A) correlations and B) loadings on each singular axis (SA). Correlations are shown for the combined data (independent of prey type), and for each prey type. ACC acceleration, DEC deceleration, PG peak gape, UJ upper jaw, VEL velocity.

Table 4.2 Variable loadings

Variables	Combined PLS	NE prey PLS	Variable exclusion PLS	Combined PCA - PC1	Variable exclusion PCA - PC1
<i>LOCO</i>					
Velocity at PG	0.408	0.430	-	0.799	0.913
Acceleration at PG	-0.211	-0.329	-	-0.585	-
Mean approach velocity	0.322	0.410	0.403	0.664	0.710
Peak velocity	0.410	0.416	-	0.923	-
Time of peak velocity	0.401	0.344	0.565	0.803	-
Peak acceleration	0.265	0.296	0.250	0.720	0.452
Time of peak acceleration	0.357	0.170	0.515	0.703	0.816
Peak deceleration	-0.278	-0.327	-	-0.742	-
Time of peak deceleration	0.285	0.155	0.437	0.558	0.669
<i>FEED</i>					
Peak gape	0.440	0.342	-	0.723	-
Time to peak gape	-0.542	-0.457	-	-0.681	-
Peak aperture area	0.251	0.235	0.378	0.434	0.022
Time of peak aperture area	0.047	0.083	0.106	0.224	0.327
Upper jaw protrusion at PG	-0.079	0.071	-	0.283	0.685
Peak upper jaw protrusion	0.109	0.244	0.239	0.586	0.824
Time of peak upper jaw protrusion	0.090	0.237	0.219	0.387	0.518
Peak gular displacement	0.384	0.382	-	0.748	-
Time of peak gular displacement	-0.051	-0.125	-0.055	-0.171	-
Peak opercular expansion	0.287	0.062	0.378	0.036	-0.532
Time of peak opercular expansion	0.107	-0.183	0.177	-0.079	-0.112
Cranial rotation at PG	0.196	0.346	0.354	0.568	0.543
Peak cranial rotation	0.176	0.293	0.312	0.642	0.607
Time of peak cranial rotation	0.327	0.295	0.583	0.556	0.225
Red: two most positive loadings, Blue: two most negative loadings PLS partial least squares, PCA principal components analysis					

$F_{1,18.7} = 18.71, p = 0.0004$), further supporting the conclusion that the two prey types used were sufficient to elicit extreme patterns of performance. Although the assumption of normality was met for all groups (Shapiro-Wilk's test, $w > 0.883, p > 0.0525$), the assumption of equal variances was not met for the FEED dataset (Levene's test, LOCO: $F_{1,29} = 0.0898, p = 0.767$; FEED: $F_{1,29} = 27.24, p < 0.0001$), indicating that feeding kinematics when capturing non-evasive prey are significantly more variable than when capturing evasive prey. A significant correlation between datasets was only observed for non-evasive prey (non-evasive: $r = 0.85, p < 0.0001$; evasive: $r = 0.48, p = 0.0616$), however the correlation coefficients were not significantly different between prey types (Steiger's $Z, z = 1.83, p = 0.0673$).

To determine if the patterns described when PLS was performed independent of prey type effectively described the patterns within prey types, PLS was also performed for each prey type. One SA was significant for non-evasive prey, however no axes were significant for evasive prey, which is likely due to the low correlation between locomotion and feeding for evasive prey as shown in the combined PLS. Within non-evasive prey, the amount of variation explained by each dataset (LOCO: 50% explained; FEED: 22% explained) and the strength of the correlation ($r = 0.86, p < 0.0001$) were similar to the combined analysis. Although this correlation was slightly stronger than in the combined analysis, it was not significantly different between tests (Steiger's $Z, z = -0.09, p = 0.9283$). Axes loadings were similar for locomotion but varied slightly for feeding (Table 4.2). However, interpretations were similar to the combined analysis.

Therefore, the combined analysis effectively captured the patterns of integration within each prey type.

Finally, to determine the sensitivity of PLS results to the inclusion of variables, The four variables with the strongest loadings (2 positive and 2 negative) on each axis (Table 4.2, Figure 4.5B) were removed and the combined PLS was re-run. Peak deceleration, acceleration at peak gape, peak velocity, and velocity at peak gape were removed from the LOCO dataset and time to peak gape, upper jaw protrusion at peak gape, peak gape, and peak gular depression were removed from the FEED dataset. Again, only one axis was significant, the amount of variation explained by each dataset (LOCO: 52% explained; FEED: 8% explained), the combined correlation ($r = 0.69, p < 0.0001$), and the correlations within each prey type (non-evasive: $r = 0.70, p = 0.0034$; evasive: $r = 0.40, p = 0.1285$) with these variables removed were similar to the inclusive analysis. Although the correlations were weaker, they were not significantly different than the inclusive analysis (Steiger's Z , combined: $z = 1.27, p = 0.2041$; non-evasive: $z = 0.95, p = 0.3421$; evasive: $z = 0.25, p = 0.8026$). Finally, the variable exclusion PLS demonstrated similar interpretations of axes loadings, despite slight differences in magnitudes, as the inclusive PLS (Table 4.2). Therefore, although the quantitative patterns may differ, the qualitative patterns are upheld and PLS appears robust to the patterns of integration between locomotion and feeding.

Principal components correlation

Principal components analysis (PCA) has also been used as an alternative method to PLS to summarize each dataset and perform correlations. Therefore, PCA correlations were also performed for comparison to PLS correlations. PCA was performed on the LOCO and FEED datasets separately, and the first principal component (PC) axes from each dataset were correlated. The first PC for LOCO explained 53% of the variation in locomotor kinematics, and 7 PCs significantly explained 99% of the variation (Bartlett's test, $p \leq 0.0027$). The first PC for FEED explained 25% of the variation in feeding kinematics, and 7 PCs significantly explained 88% of the variation (Bartlett's test, $p \leq 0.0339$). Therefore, PCA results in a large number of axes compared to the single SA from PLS analyses. The correlations recovered were weaker than those determined using PLS (combined: $r = 0.59$, $p = 0.0005$; non-evasive: $r = 0.80$, $p = 0.0003$; evasive: $r = -0.05$, $p = 0.8501$) but were not significantly different (Steiger's Z , combined: $z = 1.91$, $p = 0.0561$; non-evasive: $z = 0.39$, $p = 0.6965$; evasive: $z = 1.46$, $p = 0.1443$). As in PLS correlations, integration was supported for the combined analysis and when capturing non-evasive prey, but not when capturing evasive prey (Figure 4.6A). Although the loading magnitudes (Table 4.2) resulted in variables loading in a different order along each axis, the pattern that each axis describes a gradient from weak to strong performance (as described with PLS loadings) is recovered (Figure 4.6B). For these reasons, although the magnitude of effects differed when using PCA, the conclusions were similar to PLS analyses and both tests were able to describe integration between locomotion and feeding.

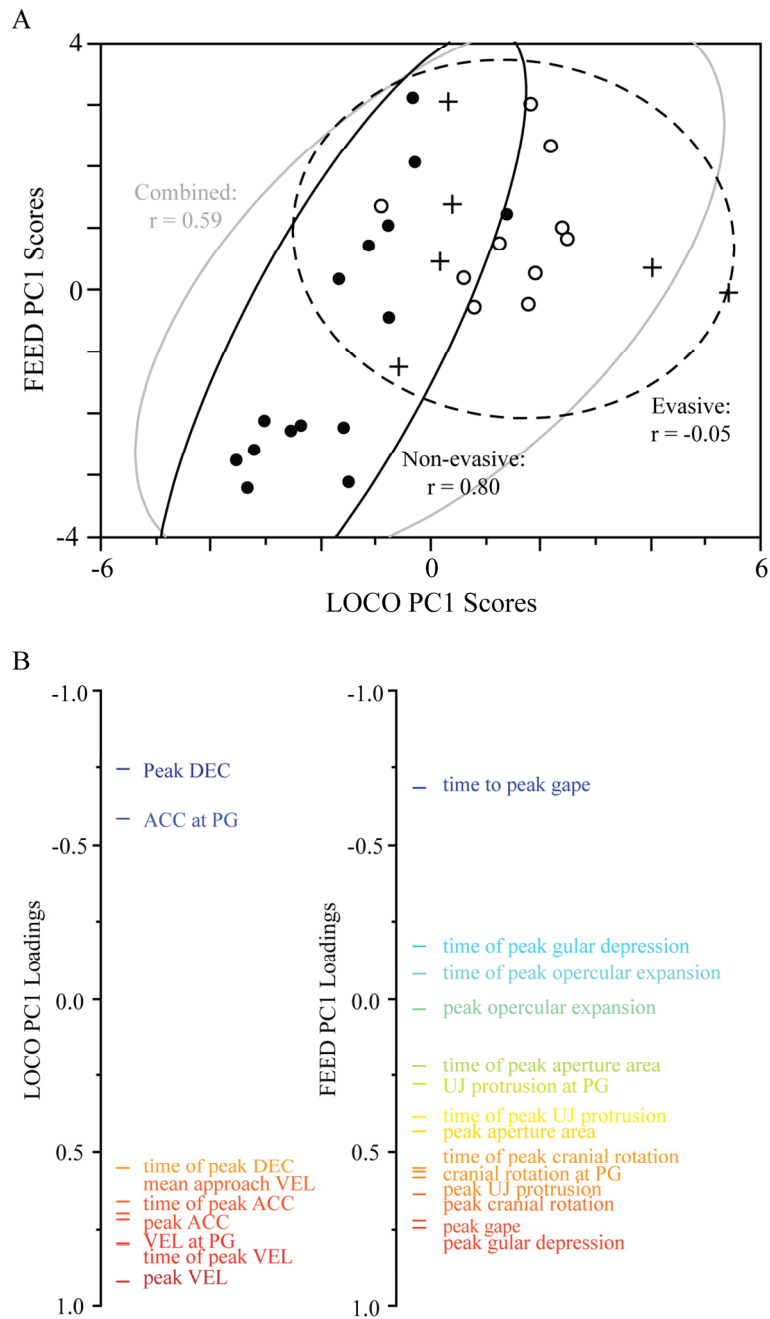


Figure 4.6 Principal components analysis A) correlations and B) loadings on each principal component (PC). Marker styles and abbreviations follow Figure 4.5.

One potential pitfall of PCA is its sensitivity to the variables included in the analysis (Carrascal et al., 2009), so as with PLS, PCA correlations were re-run excluding the variables with the top four (2 positive and 2 negative) loading along each axis (Table 4.2, Figure 4.6B). This resulted in removal of peak deceleration, acceleration at peak gape, peak velocity, time of peak velocity (LOCO), time to peak gape, time of peak gular depression, peak gular depression, and peak gape (FEED). With these variables excluded, LOCO PC1 explained 53% of the variation and 4 PCs significantly explained 97% of the variation (Bartlett's test, $p \leq 0.0161$), and FEED PC1 explained 25% of the variation and 7 PCs significantly explained 94% of the variation (Bartlett's test, $p \leq 0.0153$). Additionally, the variables that loaded strongly on each axis were similarly ordered to the inclusive PCA loadings (Table 4.2, Figure 4.6B). However, although the ordination axes were similar, the PCA with variables excluded was not able to recover a correlation in the combined dataset ($r = 0.19$, $p = 0.3177$), even though a significant correlation was observed for non-evasive prey ($r = 0.57$, $p = 0.0266$) but not for evasive prey ($r = -0.02$, $p = 0.9493$), as was determined in the PCA with all variables and the PLS analyses. None of these correlations were significantly different from the PCA with all variables included (Steiger's Z, combined: $z = 1.82$, $p = 0.0688$; non-evasive: $z = 1.1$, $p = 0.2713$; evasive: $z = -0.08$, $p = 0.9362$). This evidence suggests that PCA is not as capable of detecting the relationship between locomotion and feeding, and is more sensitive to the variables included in the analysis.

Univariate correlations

For comparison with univariate analyses of integration (Kane and Higham, 2011), univariate correlations between LOCO and FEED were performed within each prey type (Table 4.3). This analysis resulted in 126 correlations, of which 21 were significant with non-evasive prey and 6 were significant with evasive prey, suggesting that integration is greater with non-evasive prey. However, although these correlations indicate differences between prey types in the number of integrated variables, there is no way to determine whether the number of correlations within each prey type represent significant integration. Additionally, these correlations were not necessarily the variables that loaded strongly on the PLS or PCA axes. For example, evasive prey captures were located at the most positive ends of the PLS axes, driven by large gape and high velocity, but the univariate correlation of gape and velocity was not significant for this prey type ($r = 0.45$, $p = 0.0798$). The strongest correlations with non-evasive prey were observed between acceleration at peak gape and peak cranial rotation ($r = -0.68$), and time of peak velocity and peak gular depression ($r = 0.74$). For evasive prey, the strongest correlations were between time of peak deceleration and peak aperture area ($r = -0.57$), and time of peak velocity and time of peak opercular expansion ($r = 0.60$). These correlations do not align with the variables with the strongest loadings for either the combined PLS or combined PCA. Therefore, strong univariate correlations do not necessarily provide the greatest explanation of integration between datasets. Additionally, these correlations are difficult to interpret as reflecting the differences in performance indicated from both multivariate analyses.

Table 4.3 Univariate correlations between locomotor and feeding variables

	Velocity at PG	Acceleration at PG	Mean approach velocity	Peak velocity	Time of peak velocity	Peak acceleration	Time of peak acceleration	Peak deceleration	Time of peak deceleration
<i>Non-evasive prey</i>									
Peak gape	0.55	-0.38	0.53	0.49	0.58	0.25	0.00	-0.31	0.35
Time to peak gape	-0.66	0.61	-0.60	-0.65	-0.65	-0.44	-0.51	0.54	-0.30
Peak aperture area	0.38	-0.22	0.36	0.30	0.49	0.05	-0.02	-0.12	0.40
Time of peak aperture area	0.06	-0.11	0.03	0.07	0.05	-0.12	0.59	-0.09	0.31
Upper jaw protrusion at PG	0.22	-0.03	0.10	-0.07	0.22	-0.30	0.02	0.20	0.59
Peak upper jaw protrusion	0.43	-0.31	0.34	0.21	0.48	-0.01	-0.02	-0.04	0.60
Time of peak UJ protrusion	0.26	-0.36	0.23	0.26	0.53	0.34	0.06	-0.31	0.11
Peak gular displacement	0.59	-0.47	0.52	0.50	0.74	0.38	-0.07	-0.38	0.30
Time of peak gular displacement	-0.28	0.06	-0.31	-0.36	0.04	-0.21	-0.05	0.22	0.10
Peak OP expansion	0.14	0.09	0.19	0.19	0.07	0.20	0.10	-0.36	-0.46
Time of peak OP expansion	-0.26	0.17	-0.38	-0.38	-0.03	-0.05	-0.38	0.28	-0.20
Cranial rotation at PG	0.57	-0.68	0.33	0.32	0.52	0.26	0.27	-0.30	0.36
Peak cranial rotation	0.57	-0.68	0.26	0.22	0.49	0.14	0.07	-0.13	0.30
Time of peak cranial rotation	0.45	-0.45	0.30	0.32	0.42	0.35	0.37	-0.47	0.12
<i>Evasive prey</i>									
Peak gape	0.41	-0.41	0.33	0.45	0.49	0.18	0.30	-0.20	0.23
Time to peak gape	-0.02	-0.25	-0.30	-0.12	-0.19	0.02	-0.13	0.10	0.05
Peak aperture area	0.04	0.04	0.31	0.33	-0.06	0.10	-0.34	-0.21	-0.57
Time of peak aperture area	0.14	0.19	-0.17	-0.15	0.18	-0.11	0.18	0.12	-0.53
Upper jaw protrusion at PG	-0.10	0.10	0.10	-0.34	-0.41	-0.25	-0.23	0.22	-0.29
Peak upper jaw protrusion	-0.01	0.08	-0.01	-0.29	-0.15	-0.29	0.02	0.29	-0.07
Time of peak UJ protrusion	0.36	0.05	-0.07	-0.30	0.29	-0.32	0.56	0.44	0.11
Peak gular displacement	0.14	-0.08	0.06	-0.28	0.07	-0.39	0.32	0.43	0.52
Time of peak gular displacement	-0.01	-0.15	0.21	0.56	0.25	0.27	-0.02	-0.41	0.40
Peak OP expansion	-0.08	0.04	0.28	0.43	0.01	0.22	-0.27	-0.39	-0.25
Time of peak OP expansion	0.07	-0.06	-0.11	0.06	0.60	0.02	0.42	0.00	-0.17
Cranial rotation at PG	0.03	0.12	0.37	0.18	0.02	-0.18	-0.02	0.05	0.37
Peak cranial rotation	0.04	0.12	0.22	0.14	0.12	-0.21	0.03	0.12	0.26
Time of peak cranial rotation	0.35	0.26	0.06	-0.15	0.33	-0.24	0.42	0.34	0.48

Red: significant correlations ($p \leq 0.05$); PG peak gape, UJ upper jaw, OP operculum

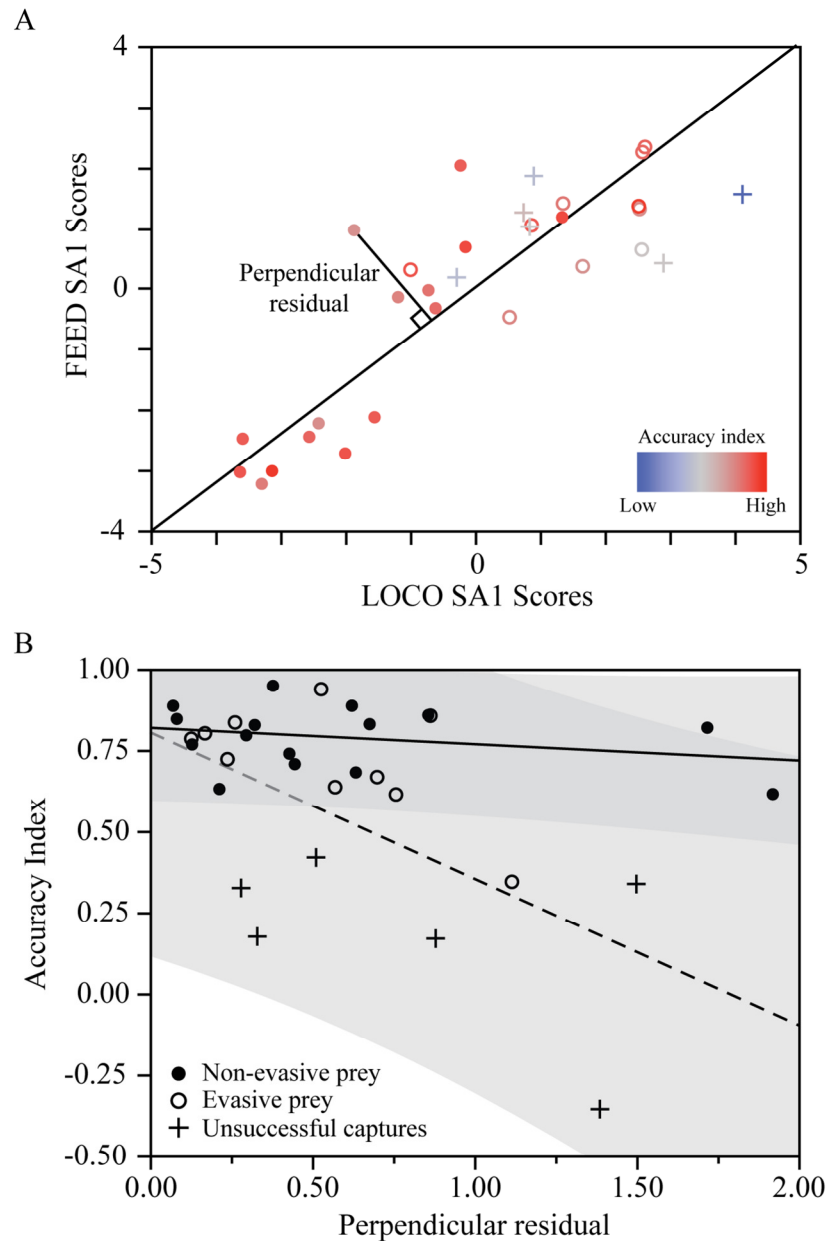


Figure 4.7 The relationship between integration and accuracy. A) PLS plot (Figure 4.5A) with accuracy index (Kane and Higham, 2014) overlain. The major axis regression line and a perpendicular residual are shown. If the regression line represents perfect integration, a large residual represents poor integration. B) Regression of accuracy index (Kane and Higham, 2014) against perpendicular residuals (departure from integration) with 95% confidence intervals of the fit shown. Scores with poor integration had low predator accuracy, but only with evasive prey; for non-evasive prey, accuracy was high for all trials, and integration had no effect.

Relationship with accuracy

Mean accuracy ($AI = 0.79 \pm 0.02$) and integration ($r = 0.85$) were both greater when capturing evasive prey compared to non-evasive prey ($AI = 0.51 \pm 0.07$; $r = 0.48$), suggesting a positive relationship between the two. The error variance ratio (ratio of variance in Y to variance in X) was 0.65 and the major axis regression (Figure 4.7) resulted in a slope of 0.808 (confidence intervals 0.623 to 1.047), intercept of 0.00, and r^2 of 0.69. The perpendicular residuals had a significant effect on accuracy both in the combined analysis and when capturing evasive prey (combined: $t = -2.16$, $p = 0.0388$; non-evasive: $t = -1.07$, $p = 0.3055$; evasive: $t = 2.54$, $p = 0.0236$). The combined regression was fit with a slope of -0.221, intercept of 0.784, and r^2 of 0.14. The evasive prey regression was fit with a slope of -0.451, intercept of 0.805, and r^2 of 0.32. This analysis supports the hypothesis that departure from integration (increased residual distance) results in poor accuracy.

DISCUSSION

Bluegill were able to coordinate locomotor and feeding performance during prey capture, but this ability differed between prey types. This general result was robust across statistical methods although the effect magnitude was weaker using PCA and univariate correlations. Traditional analyses have compared gape and velocity measures (Higham, 2007a; Higham et al., 2007; Kane and Higham, 2011; Oufiero et al., 2012), and although these variables loaded strongly on PLS axes, they were not the only ones, indicating the true multivariate nature of performance integration. Whereas timing and

magnitude of locomotor variables similarly contributed to integration, primarily magnitude of feeding variables contributed strongly to integration. Therefore, within each system, the dependence on spatial and temporal elements may vary. When capturing evasive prey, bluegill used kinematics representing higher performance in both the locomotor and feeding regimes, and these changes resulted in decreased integration. Therefore, not only does this support the non-additive nature of performance integration, it demonstrates that performance integration is dynamic compared to static measures of morphological integration. Finally, the relevance of performance integration to predatory fishes is its contribution to predator accuracy. Departure from integration, represented by perpendicular residuals to the major axis line of fit, resulted in decreased predator accuracy, which in turn has consequences on predator success (Kane and Higham, 2014). Therefore, when feeding kinematics are paired with locomotor kinematics in a coordinated way, predators increase their chance of positioning themselves in an ideal location relative to the prey. These results are important for establishing a foundation upon which further questions of performance integration can be asked.

Performance integration in the context of previous studies

The idea of coordination among parts of an organism has been prominent in biology for over 150 years, when Charles Darwin emphasized the significance of “correlated growth” in organisms (Darwin, 1859). Darwin saw integration as the correlation between two phenotypic characters, such as deafness and blue eyes in cats, and recognized that this interdependence can function independent from (but subordinate to) natural selection

as a source of evolutionary change (Darwin, 1859 p. 143; Darwin, 1868; Gould, 2002 p. 332-341). More recently, integration has been used in a diversity of applications, where it has taken on the simplified meaning of “to combine” (e.g. Vitousek, 1990; Liem and Summers, 2000; Levit et al., 2004). Integration has also been used synonymously with organismal complexity (Olson and Miller, 1958; Wake and Roth, 1989), where increasing complexity necessitates parts working together to form a whole (Dullemeijer, 1974). The idea of integration as a statistical correlation became prominent nearly 60 years ago, when a series of univariate correlations were interpreted as defining integrated “sets” of traits (Olson and Miller, 1951; Olson and Miller, 1958) or “correlation pleiades” (Berg, 1960). In a similar way, recent literature defines integration as the correlation of traits within functional modules (Cheverud, 1982; Zelditch et al., 2004; Klingenberg, 2008), and often the emphasis is on integration of morphological traits such as skull shape (Roseman et al., 2011; Cáceres et al., 2014). It has not been until the most recent decade that performance integration was suggested as an area of research on its own (Higham, 2007b; Higham, 2007a). Whereas this previous work had only considered static integration of structural parts, performance integration is dynamic, and the magnitude of integration can change each time the behaviors are performed.

Recently, several approaches have been used to understand how functional traits interact within and across organisms in the context of performance. One approach was first outlined by Lauder (Lauder, 2000a) and later applied to understand interactions between morphology, mechanics, and kinematics in running lizards (McElroy and Reilly, 2009). In this method, each performance regime was summarized with an ordination, the

ordinations were then stacked, and lines connecting groups between layers were used to visualize the interactions between performance spaces. However, this technique is primarily qualitative. More recently, quantitative genetic methods have been adapted to understand how the relationship between morphological and performance traits affects phenotypic evolution among populations (Ghalambor et al., 2003; Walker, 2007). Here, standard partial regression coefficients of performance traits on morphological or physiological traits are used to quantify the interaction of morphology on performance within the context of a larger model. However, this method is based on multiple regression, where predictors are assigned coefficients that explain their relationship to the response with all other predictors held constant, and this method may not be relevant for integration (Zelditch et al., 2004). A logical extension of this idea to performance integration would be to ask how the relationship between two performance systems affects phenotypic evolution, were the morphological or physiological traits could be replaced by an additional set of performance traits. Therefore, the general idea of assessing constraints on the ability for organisms to evolve could be useful for future work assessing consequences of performance integration, and this technique could become adapted for those questions.

Quantitative methods to assess the integration of traits more directly have ranged from measures of repeatability to univariate and multivariate correlations, but none of these methods have been able to directly assess the covariation itself. Work assessing the coordination between feeding, locomotion, and vision during prey capture compared species by qualitatively assessing the timing of movements and using coefficient of

variation to signify repeatable, or highly coordinated, movements (Rice and Westneat, 2005; Rice et al., 2008). However, coordination within each variable was considered independently, and the interactions among variables were not quantified. A large amount of work on performance integration has relied on univariate correlations, either between morphological variables with direct relationships to performance (Higham, 2007b), between velocity and gape as representative performance measures (Higham, 2007a; Higham et al., 2007; Oufiero et al., 2012), or between multiple performance measures (Kane and Higham, 2011). But as demonstrated with the current work, univariate correlations may not be important for describing covariation, and do not fully capture the level of integration among performance measures. Additionally, interpreting the relevance of the correlations, assessing the overall level of integration within a group, and determining differences between groups using a series of univariate correlations is difficult.

Others have also recognized these pitfalls with univariate analyses of integration, and have used multivariate ordination techniques to either describe the patterns of variation within multiple levels of performance, where variables that loaded on the same axes were considered coordinated (Rice, 2008), or to summarize functional morphological or performance datasets, where correlation or regression of axes represent integration (Collar et al., 2008; Montuelle et al., 2009; Montuelle et al., 2012a). Although these methods are an advancement over univariate analyses, the ordinations did not directly assess the covariation between performance datasets. Therefore, the current study represents an advancement over previous methods because the relationship between

performance datasets was used in the ordination itself. However, because PLS and PCA techniques recovered similar interpretations, at least within the context of the current data, the axes of variation appear to be similar to the axes of covariation, indicating that previous ordination-based analyses may be adequate to describe performance integration in those systems. However, because PLS directly assesses the relationship between performance datasets, and was shown here to be more robust to variable removal, it should be considered as a primary analytical tool for future analyses of performance integration.

Integration of locomotion and feeding performance in Bluegill

For the first time, this study demonstrated that multivariate performance of the locomotor system was correlated with multivariate performance of the feeding system in bluegill, and that both kinematic performance and integration were modulated across prey contexts. Although multivariate integration was expected based on previous work (Higham, 2007a), it is possible that additional variables could be performed randomly, leading to an inability to determine a pattern and a lack of multivariate integration. This alternative was not supported, suggesting that bluegill locomotor and feeding kinematics are coordinated in a multivariate way. Within prey types, stronger integration may be expected when capturing evasive prey because higher attack speeds and limited duration of the capture event have resulted in increased coordination in cichlids and serranids (Liem, 1978; Oufiero et al., 2012). Although higher attack speeds and shorter durations were observed for evasive prey compared to non-evasive prey in bluegill (Kane and

Higham, 2014), integration was weaker. Because bluegill are suction specialists (Carroll et al., 2004; Collar and Wainwright, 2006) that approach prey at low speed (Higham, 2007a), large evasive fish prey may be more challenging to capture, resulting in more random attack kinematics and a lack of integration. Alternatively, bluegill may approach a performance plateau that limits the ability to coordinate behaviors (discussed below). Bluegill demonstrated the strongest integration when capturing non-evasive prey. Since this prey type permits use of specialized suction behaviors, bluegill may be better able to integrate performance across behaviors. However, non-evasive prey were intended to elicit low performance (in contrast to attached prey which are non-evasive but elicit high suction performance (Holzman et al., 2012)), where prey could be successfully captured with a variety of behaviors and integration may not be necessary. Therefore, the result that bluegill demonstrate strong integration on this prey type suggests little cost of integration to the predator when capturing non-evasive prey, and strong integration may be a default behavior in bluegill. This supports the hypothesis that a correlation between locomotion and feeding will be strong in specialists (Webb, 1984b), presumably when they use the specialized behaviors.

This study also provides evidence that some feeding contexts may impose constraints on the ability to integrate performance. Specifically, integration was stronger when capturing non-evasive prey than evasive prey, and this lack of integration may be due to bluegill reaching their performance limits. The LOCO and FEED PLS axes represented performance gradients, with high performance behaviors associated with positive values. Therefore, when capturing non-evasive prey, bluegill used the full range of feeding

performance, but a constrained, lower range of locomotor performance. Alternatively, when capturing evasive prey, bluegill used only the higher range of both feeding and locomotor performance. Since bluegill are suction specialists that primarily capture small evasive or attached prey (Carroll et al., 2004; Collar and Wainwright, 2006; Holzman et al., 2012), then large evasive prey such as other fishes likely evoke more unspecialized behaviors at the edge of their performance capabilities. Therefore, this may have limited their ability to integrate behaviors.

Further evidence of a performance limit is given by the major axis of the trials for each prey type. Although this was not formally analyzed, qualitatively it is apparent that non-evasive prey would have a steeper slope than evasive prey. Therefore, although changes in locomotor performance evoke large changes in feeding performance with non-evasive prey, changes in feeding performance are constrained with evasive prey. This suggests a feeding performance limit is reached, leaving changes in locomotor performance as the primary means of modulation. Whereas mouth expansion is limited by the size of the head and jaws, movement of the body toward the prey may be more flexible because multiple control surfaces (e.g. pectoral fins) can be recruited to increase performance. However, bluegill tend to be more specialized for maneuvering, low velocity locomotion (Webb, 1984b; Webb, 1984c; Collar and Wainwright, 2009), and when using high velocity and acceleration behaviors to capture evasive prey bluegill simply may not be able to maintain integration. If bluegill are indeed encountering a performance limit with evasive prey, it is possible that integration is non-linear, such that a plateau is reached at high levels of locomotor performance. This is a novel result in

organismal integration, and it may be possible that non-linear patterns are only apparent when integration is dynamic and flexible within an organism, such as with performance. Therefore, performance integration represents an important and understudied research field that can provide novel insights into organism performance, and should be given increased attention in future studies.

Another important result from this study is that while multiple performance variables contribute to integration across bluegill prey capture attempts, the importance of these variables may be weakened as bluegill reach their performance limit. The strongest variable loadings for locomotion and feeding were velocity at peak gape and peak gape, respectively, and these strong loadings support their use in previous studies of integration (Higham, 2007a; Higham et al., 2007; Oufiero et al., 2012). However, if integration weakens at the highest levels of performance, this indicates that dependence on these two variables weakens as well. Following this logic, there may actually be an intermediate point where integration is strongest and velocity and gape have the greatest effect. Therefore, ram and gape drive the relationship between locomotor and feeding performance, but their importance may be weakened in some contexts. This further supports the observation that high locomotor performance is incompatible with high feeding (suction) performance (Higham, 2007a) since bluegill are suction specialists (Carroll et al., 2004; Collar and Wainwright, 2006).

The influence of predator strategy on integration

This study only examined one species, and it is possible that the patterns observed here may not be supported in other species with divergent predatory strategies. The four primary feeding modes described in fishes are suction, ram (overtaking prey without suction), biting (removing pieces of prey), and filter feeding. Within suction, fish can either generate fast water flow and strong forces by coordinating the timing of the feeding movements (Holzman et al., 2007; Bishop et al., 2008), or generate large ingested volumes by modulating the magnitude of mouth expansion (Higham et al., 2006). Additionally, fishes can approach prey using low velocity maneuvering (e.g. in complex habitats (Morice et al., 2013)) where deceleration may be important (Higham, 2007a), high velocity pursuit where sustained velocity may be important (Nyberg, 1971; Webb, 1982a; Webb, 1984a), or rapid ambush attacks where acceleration may be important (Webb and Skadsen, 1980; Webb, 1982a; Webb, 1984a). Therefore, specializations along any of these dimensions could affect the ability to integrate behaviors. Additionally this diversity in approach and capture strategies suggests that the relevance of performance variables may change with these specializations and that predator strategy can have a large influence on the strength and composition of performance integration.

To start to understand how integration varies with ecological differences among species, closely related species with divergent strategies should be examined. A previous study used this approach to understand differences in predator accuracy and success (Kane and Higham, 2014), and the same species could be used to understand interspecific variation in performance integration. That study used bluegill, a species that uses forceful

suction in combination with deceleration (likely for maneuverability), largemouth bass (*Micropterus salmoides*), a species that uses high volume suction and relies on high velocity pursuit, and green sunfish (*Lepomis cyanellus*), a species that also relies on high volume suction but is a high acceleration ambush predator. Like bluegill, bass were able to modulate the ingested volume of water (IVW), suggesting that differences in integration between prey types might be expected. However, unlike bluegill, the strongest integration might occur when capturing evasive prey because bass are specialized for this prey type (Collar et al., 2005; Collar et al., 2009). Additionally, increased variation in IVW shape when capturing non-evasive prey suggests that a similar pattern may be observed in performance variables, and that integration may be weak as a result. Alternatively, although green sunfish converge on piscivory (Collar et al., 2009), this species did not modulate the IVW, suggesting that performance is similar across prey types and that integration would also be similar. Additionally, since accuracy and success with evasive prey were lower for green sunfish than for bass, this suggests that performance integration might also be weaker than bass, resulting in poor success. Compared to bluegill in the current study, bass and green sunfish would not be expected to reach a performance limit because not only are their mouth sizes larger (Carroll et al., 2004; Collar and Wainwright, 2006; Kane and Higham, 2014), they also exhibit specializations for high velocity and acceleration performance (Webb, 1984b; Webb, 1984c). The greater capacity for both feeding and locomotor performance in these species suggests that they may be more flexible across prey types than bluegill, and that a potential cost of integration to bluegill is a diet restricted to smaller, less responsive prey.

Conclusions

Coordination can be due to cause and effect relationships, mechanical linkages, or co-dependence of systems (Wainwright et al., 2008). Fishes provide examples of all three of these mechanisms (Bainbridge, 1963; Westneat, 1990; Sfakiotakis et al., 1999; Durie and Turingan, 2004; Lauder and Tytell, 2005; Higham, 2007a; Higham, 2007b; Higham et al., 2007), and for the first time, co-dependence between locomotor and feeding performance has been quantified in a multivariate context. This co-dependence has been demonstrated as a mechanism contributing to increased predator accuracy during prey capture on evasive prey, and therefore provides relevance of integration in an ecological context. Whereas cause-effect relationships and mechanical linkages directly result in coordination, the combined use of two systems that perform different tasks can result in varying degrees of coordination, and therefore this mechanism can be considered indirect. Because these indirect mechanisms are more difficult to quantify and assess, this area of research remains relatively unexplored. By providing an example where integration has been demonstrated within and across varying conditions, the kinematics driving integration have been assessed, and the ecological relevance has been supported, these studies provide the foundation for making locomotor and feeding integration in fishes a model system for understanding the emergent level of performance that results from coordination and co-dependence of systems that perform different tasks.

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CHAPTER 5:

Conclusions

While it is generally understood that organisms are complex, most approaches to organismal function are reductionist and attempt to understand function within parts rather than across parts (e.g. Losos and Sinervo, 1989; Bels et al., 1997; Domenici and Blake, 1997; Wakeling and Johnston, 1998; Drucker and Lauder, 1999; Motta et al., 2002; Sanford and Wainwright, 2002; Carroll et al., 2004; Lauder and Tytell, 2005; Van Wassenbergh et al., 2005; Blake, 2006; Higham et al., 2006; Deban et al., 2007; Mehta and Wainwright, 2007; Ferry-Graham et al., 2008; Roos et al., 2009; Combes et al., 2010; Kane and Higham, 2012; Camp and Brainerd, 2014). These studies have provided tremendous advances in the way we understand organisms, and because of those advances, this is an optimal time to begin to think about how those parts interact within organisms. Because integration among functions is an emergent property of functional systems that is not apparent unless these interactions are examined, integration provides a novel way to understand how organisms work. The work presented in this dissertation is transformative because it offers a new way to analyze and interpret common behaviors that can lead to novel insights into organism function. By providing new methods and an ecologically relevant examination of performance integration, the tools are now readily available for other researchers to apply these ideas to their own work.

Both the physical and life sciences are increasingly focused on explaining natural complexity, or the variation in interactions among parts resulting in unpredictable

emergent properties (discussed in Solé and Goodwin, 2000). Physicists have shown that complexity in natural systems is often self-organizing until it reaches a critical point where organization fails (Bak et al., 1988; Camazine, 2003). This idea can be demonstrated by the formation of a pile of sand (Bak et al., 1987; Bak, 1996). As sand is poured onto a flat surface, a pile develops and is an emergent property of the sand grains. Once the pile is large enough and the sides are sloped a critical point is reached and grains begin to slide down the pile until the pile becomes too large and collapses. In this way, the sand grains self-organize into a pile that stabilizes at a critical point characterized by frequent small failures (sliding), but occasionally those small failures cause infrequent large failures (collapse). Self-organized criticality has been used to explain phenomena as diverse as earthquakes, species extinction, and economic crashes (Bak, 1996; Solé and Goodwin, 2000; Camazine, 2003). Criticality has even been implicated to work in conjunction with natural selection in the evolution of organisms (Solé et al., 1999; Camazine, 2003). Therefore, self-organized criticality (Bak et al., 1988; Jensen, 1998) and interactions among parts of a system may be an important and overlooked process driving organismal function.

Complexity has been suggested as a major scientific frontier in behavioral ecology (Bradbury and Vehrencamp, 2014) and it is not unreasonable to think the same could be true for studies of organism function. Performance integration is an emergent property of movement of the parts comprising the locomotor and feeding systems. Therefore, integration may be the result of self-organization, where interactions between the movements govern how they can be used together. The number of movements performed

simultaneously may result in small failures that happen often (poor accuracy), but may occasionally result in large failures (poor success). It is also likely that criticality may be different in different contexts, such as with different prey items. In this way we may be able to understand performance integration in the context of deeper physical principles such as complexity and self-organization.

One explanation for the distribution of organisms across habitats has traditionally been in the context of function and is explained using an adaptationist view (Gould and Lewontin, 1979) of mechanical principles. For example, organisms are thought to survive better in certain habitats because the ability to move or feed in those habitats presents an advantage over other species (e.g. Arnold, 1983; Langerhans and DeWitt, 2004; Walker et al., 2005; Calsbeek and Smith, 2008). However, given the role of complex interactions between parts in regulating organization and function, it is possible that performance integration can provide an alternative explanation of organism survival – some animals may simply be better able to survive because the interactions among performance traits are less prone to large failures. For this reason, complexity may be a frontier not just in behavioral ecology, but rather more generally in organismal biology, and this dissertation is a first step in exploring this new frontier.

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