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A Longitudinal Assessment of Temperament in *Octopus bimaculoides*

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The abstract and thesis of David Loyd Sinn for the Master of Science in Biology were presented April 19, 2000, and accepted by the thesis committee and department.

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ABSTRACT

An abstract of the thesis of David Loyd Sinn for the Master of Science in Biology presented April 19, 2000.

Title: A Longitudinal Assessment of Temperament in *Octopus bimaculoides*

Cephalopods, including *Octopus* spp., are highly intelligent molluscs that play major roles in many marine food webs, both as top-level feeders (Ambrose, 1984) as well as by constituting a major source of protein for the animals above them (Lang, Hochberg, Ambrose, & Engle, 1997). They also are fascinating organisms for behavioral studies, with elaborate repertoires of behavior based on plasticity and learning (Wells, 1962a; 1962b; 1978) which in complexity rivals that of the vertebrates. The study of individual differences in behavior is a facet of behavioral research that has recently gained attention in the literature (Gosling & John, 1999). Traditionally, behavioral studies previously have been largely based on characterizing groups of animals at the level of the population or species (Slater, 1981). The study of individual variance has risen in importance, however, as we have begun to realize how behavior at the level of the individual contributes to the shaping of the ecological profile of a population (Wilson, Coleman, Clarke, & Biederman, 1993).

Octopus bimaculoides (Pickford & McConnaughey, 1949) offers a previously unstudied model of invertebrate individual differences. Individual differences at a young age are considered to be components of an individual's temperament (Rothbart, Ahadi, & Young, 2000), which are behavioral trait dimensions researchers use to

describe the traits upon which individuals differ (Buss & Plomin, 1984). Since temperament has not been defined previously for this species, this study first describes temperament at week 3 of life in *O. bimaculoides*. Secondly, no longitudinal studies have been performed to examine the development of these traits within an invertebrate. The second aspect of this study then examines the development of temperament through week 9 of life. Throughout both aspects of this study, the role of inheritance in these behaviors as well as the analytical methodology used in the study of individual differences is stressed.

This study attempts to satisfy some of the need for systematic behavioral development studies in *Octopus*, while also presenting to those readers of psychology and behavior the first invertebrate model of temperament.

A LONGITUDINAL ASSESSMENT OF TEMPERAMENT IN *OCTOPUS BIMACULOIDES*

by

DAVID LOYD SINN

A thesis submitted in partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE
in
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Dedication

This work is dedicated to four people: Dr. Leonard Simpson, Ronald Sinn, Judith Sinn, and Margaret Wolszon. This work truly would not have been possible without each of you. Thank you.

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This was not a single person project, and there were many that played important roles in the completion and support of this work. If there are any that I have left off, I apologize for the oversight.

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I would like to thank the Biology Department at Portland State University for space and teaching assistantships, as well as the Hawaiian Malacological Society and the American Malacological Society, both of which provided grant money that was sorely needed.

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"What is needed is, first, a description of the changes that take place during the life of the individual under natural circumstances, and second, an analysis of the factors that underly the observed changes."

- J. P. Kruijt, 1971

"The subjective experience of an animal, if it has any, may be totally different from humans, reflecting its different way of life and the different ways in which its body works."

- P. Martin & P. Bateson, 1993

General Introduction

Cephalopods, which include *Octopus* spp., are generally regarded as intelligent marine invertebrates with intricate sense organs, large brains and complex behavior (Wells, 1962a; 1978; Young, 1964; 1971; Packard, 1972; Mather, 1995; Hanlon & Messenger, 1996). Their life history as soft-bodied invertebrates coupled with their complex behavioral repertoires make *Octopus* fascinating marine organisms for ethological research. Individual differences in the behavior of octopuses are one aspect of ethological study that has thus far been only briefly touched upon in the literature (see Mather & Anderson, 1993, for a description of individual difference in adult octopuses). The study of the formation and expression of relatively stable individual differences in behaviors is important since these differences, much like physical traits, provide material for selection (Slater, 1981; Clarke & Boinski, 1995). This work combines the need for systematic behavioral studies in octopuses along with the examination of the development of behavioral polymorphism in an invertebrate by focusing on early individual differences in the behavior of young *Octopus bimaculoides* (Pickford & McConnaughey, 1949).

The main body of this work is separated into two chapters. The first defines temperamental dimensions at three weeks of age while the second examines the development of these trait dimensions through the first nine weeks of life. Both chapters stress the influence of relatedness upon the expression of these traits as well as the statistical methods used in generating these results. Temperament was chosen as the conceptual framework for both chapters, since temperamental differences are those thought to best represent individual differences in behavior of young organisms (Buss & Poley, 1976). Thus, a brief section that provides a framework for this theory is included in the general introduction, along with background information on *O. bimaculoides* and factor analysis, an analytical tool used in the study of individual differences.

A general conclusions section following Chapter 2 summarizes the findings of both thesis chapters, suggests avenues for further research, and also makes suggestions to cephalopod researchers who are interested in taking the individuality of their subject organisms into account. Finally, two additional appendices are given in order to document the culturing methods used during experimentation and to provide a general report of observations on the first occurrences of behaviors in *O. bimaculoides*.

Temperament and Individual Differences

Temperament provides the conceptual basis with which to study individual differences at an early age in organisms since these traits are thought to be biologically based behavioral characteristics that appear shortly after birth (Buss & Plomin, 1984). By biologically based I mean that these traits represent genetically transferable biases towards particular behavioral styles, with innate behavioral and physiological mechanisms providing the basis for genetic transfer of information (Goldsmith, et al., 1987; Gunnar, 1987; Fox, 1989; Kagan, 1989; Kagan & Snidman, 1991). Temperament can be conceptualized as behavioral *styles* rather than discrete behavioral *acts* (Thomas & Chess, 1977) and are broad, stable, relatively transituational behavioral continuums that can be used to characterize and rank individuals relative to one another (Buss and

Poley, 1976).

How can temperament traits be measured? In practice, temperament can be measured through behavioral responses to novel or stressful situations. Infant and young individual response styles are considered to be more accurate indicators of temperament than are adult measurements, when coping mechanisms are thought to be more well-developed and learned responses may contribute to the observed variation (Thomas & Chess, 1977). Factor analysis provides the analytical tool by which researchers define temperament dimensions; trait dimensions that arise through this analysis characterize a number of individuals, but individual differences can be observed in the amount or level of expression of these traits (Cattell, 1965). Temperament, therefore, is a behavioral model that can be used to characterize individual differences in very young organisms (Thomas & Chess, 1977).

How can octopuses contribute to our knowledge of temperament theory? Initial precedence for these types of studies comes from secondary descriptions in the literature describing high variability in individual octopus responses (Wells, 1962a; Allen, Michels, & Young, 1986). Bradley (1974) attributed observed behavioral variability of wild-caught *O. vulgaris* to previous individual experience in the sea, noting that individuals were different due to their developmental learning experiences, a hypothesis also suggested more recently by Mather & Anderson (1993) in their work with adult *O. rubescens*. Thus, for at least thirty years researchers have noted both the high variability between different octopuses as well as the stability within individual octopus responses in their studies.

In addition to their observed individual distinctiveness, *Octopus* spp. bring certain unique features to the study of individual differences when contrasted with that of vertebrates previously studied for these traits. While octopuses are on their own immediately upon hatching, experiencing no postnatal care from the parents whatsoever (Boletzky, 1987), many of the vertebrates previously studied for temperament traits have been mammalian, which do undergo periods of parental care. These differing early experiences provide differing constraints driving the development of early acceptable behaviors, and each group's early behavioral strategies should reflect their respective developmental context. The early life of the octopuses is thought to be characterized by a very early behavioral plasticity coupled with a heavy reliance on learning (Wells, 1962a; 1962b; 1978), in order to ensure survival of the solitary young. Although some of these same mechanisms must exist in the mammals, the fact that there is no generational overlap among the octopuses (Hanlon & Messenger, 1996) should require that their behavioral strategies regarding early survival are different. Other life history features, such as the demands of the marine environment compared to that of a terrestrial one, should also contribute to unique features of behavioral style across these two groups. The unique formation of cognitive traits between these two groups in regards to their respective environments has already been proposed for the formation of intelligence (Humphrey, 1976; Robinson, 1990). The octopuses differing, yet equally complex, behavior and life history provide an excellent alternative model to that of the vertebrates from which to study the development of individual differences.

Octopus bimaculoides

Octopus bimaculoides (Cephalopoda: Octopoda: Incirrata) is one of approximately 150 known members of the genus *Octopus* (Nesis, 1987). The known geographic range of *O. bimaculoides* is from central California (San Simeon) to central Baja (Guerrero Negro), at depths less than 20m (Roper, Sweeney, & Nauen, 1984; Forsythe & Hanlon, 1988a; Lang, 1997). Its known habitats are variable, including rocky reefs, giant kelp forests, sandy bottoms, and mud flat environments (Lang, 1997). The known life span of *O. bimaculoides* in its natural habitat ranges from 12 to 18 months (Forsythe & Hanlon, 1988b).

Although little is known about many aspects of the life history and behavior of this organism, descriptions in the literature have begun. Early studies included the original description of the species (Pickford & McConnaughey, 1949) and of their mesozoan kidney parasites (McConnaughey 1941, 1951, 1960). Peterson (1959) also gave an early description of the anatomy and histology of their reproductive system. More recently, their general anatomy has been described (Stoskopf & Oppenheim, 1996) and knowledge concerning their ecology summarized (Hochberg & Fields, 1980; Lang, 1986, 1997). Forsythe, DeRusha, & Hanlon (1984) and Forsythe & Hanlon (1988b) reported the first results of culture experiments with these animals in the laboratory. Two general, non-systematic descriptions of behavior in *O. bimaculoides* have been given by MacGinitie & MacGinitie (1968) as well as by Forsythe & Hanlon (1988a). Boal (1991, 1996) has largely provided the current knowledge of learning constraints in this species, while Cigliano (1993) described dominance hierarchies formed in the laboratory under limiting resource conditions. Skin patterning in this species has also been described (Packard & Hochberg 1977; Forsythe & Hanlon, 1988a).

While knowledge of the behaviors of the young of any *Octopus* spp. is minimal, a few studies have described broad, general behavior patterns (Messenger, 1963; Wells and Wells, 1970; Mather, 1984; Nixon, 1985; Boletzky, 1977; 1987), but only one has addressed *O. bimaculoides* (Forsythe & Hanlon, 1988a). Certain developmental aspects have been studied in cephalopods, including activity cycles (Mather, 1984), feeding (Messenger, 1973), and overall observations (Wells & Wells, 1970; Hanlon, Turk, Lee, & Yang, 1987). In general, however, we know little about the development of behavior in this group (Hanlon & Messenger, 1996). To date, no studies have been performed to quantify temperamental dimensions within any octopus. This study provides the first report of broad behavioral tendencies at an early age in this group as well as the first report of the development of these tendencies through the early life. This work also represents one of the first systematic, quantitative descriptions of the development of behavior in any *Octopus*.

Factor Analysis

Factor analysis is the statistical and analytical tool most often used by behavioral researchers for the study of early individual differences. Factor analysis allows us to take quantifiable behavioral data and search for inferences concerning the underlying correlations among the behaviors themselves (Cattell, 1965; Buss and Poley, 1976). It explains the patterns of correlations among multiple variables by finding a smaller number of factors than variables that account for a large proportion of the variance among the original measurements (Tabachnick & Fidell, 1996). It is the tool chosen here to analyze the behavioral data taken from *O. bimaculoides*.

In carrying out a factor analysis, data are gathered on a number of individuals for a number of behavioral or psychological variables. The resultant factors, then, suggest hypotheses about the communalities among the original multiple variables since the factors represent the underlying influence of the variables, or the behaviors themselves (Buss & Poley, 1976). *Nota bene:* 'Communality' is the term used to describe underlying relationships of variables in factor analysis. The factors that are obtained through this analysis are considered primary dimensions of individual differences, with each dimension representing a distinctly different source of variation causing individual differences in the behavior of living organisms (Cattell, 1965). Desirable properties for a factor solution include parsimony in the number of factors explaining most of the variance and conceptual meaningfulness of the factors as they relate to the variables (Comrey & Lee, 1992).

Two matrices are obtained when performing factor analysis. The first is a general correlation matrix, which correlates all variables with one another. Theoretically, these relationships among variables also represent the influence of the underlying common factors, but at this stage of analysis the researcher does not know what the factors are. Thus, a second matrix, or factor matrix, is computed from this first matrix and relates the variables to the underlying factors or dimensions. This factor matrix is based on the original correlations among the variables, with the relationship among the *n* variables now being expressed in terms of *k* factors. A mathematical rotation is then performed upon this factor matrix in order to spread the variance of the solution across the factors, thereby increasing the interpretability of the findings (Rummel, 1970). Rotation can be performed in one of two ways, either orthogonally or obliquely, and the choice of rotation includes assumptions concerning the structure of the factors in the study organism. Orthogonal rotation mathematically forces the factors to be unrelated to one another, and thus assumes that the hypothetical factors are also completely unrelated to one another within the psyche of the subject organism. Oblique rotation allows correlations among the factors, within a mathematical and theoretical framework (Tabachnick & Fidell, 1996). In either case, rotation is performed in a factor space in order to maximize the low and high loadings of the variables, or behaviors, on the given factors. It is this rotated matrix, or pattern matrix, that is the final matrix obtained in factor analysis (Rummel, 1970).

The overall pattern of the magnitudes and directions of the loadings of each behavior in the pattern matrix gives us an interpretation of a factor, in other words, its psychological meaning (Buss & Poley, 1976). In particular, we look at the variables with the largest loadings on a given factor and ask what it is that these variables may have in common with one another, communalities that are not present for those variables with low loadings along that same factor. Interpretation and naming of the factors requires extensive background knowledge of the data, of the behaviors performed, and of the subjects being analyzed.

Ford, McCallum, & Tait (1986) suggested strategies researchers may use to report their factor analysis to facilitate replication of results. These suggestions were followed here and include the reporting of the full pattern matrix, eigenvalues along the first ten eigenvectors, and details concerning research choices made during factor analysis.

In summary, factor analyses can be used to study temperament since: a) factors can be thought of as primary hypothetical constructs that reflect patterns of behavior based on the data, and b) factors are sources of individual differences which may explain differences in the behavior of living organisms (Cattell, 1965; Buss & Poley, 1976).

Potential Contribution of this Study

In a larger sense then this work contributes to an answer to the following questions: What behavioral tendencies are individual octopuses born with, how do these tendencies develop, and is there an influence of an individual's genetic background in these processes? More work is needed before these questions can be completely answered, but identifying early differences in *O. bimaculoides* and their patterns across the first nine weeks of life is a first step. This study is also an attempt to integrate the comparative psychology of this invertebrate with current temperament trait theory and assessment. This work should be of value both to animal behaviorists and to students of cephalopod research, as the former look to ways to assess the individual distinctiveness of their subject organisms in order to better understand them as groups and the latter strive to systematically describe behavior in this fascinating and complex group of molluscs.

Chapter 1 -Temperament in young *Octopus bimaculoides*

Introduction

Individual differences in the behavior of young organisms are described as different levels of expression of temperament traits (Rothbart, Ahadi, & Young, 2000). Temperamental traits are defined in humans as behavioral styles or tendencies (rather than discrete behavioral acts) that show continuity over time, can be identified in early infancy, and have a heritable basis (Goldsmith, et al., 1987). Temperament, therefore, provides the framework that allows researchers to define the behavioral traits in which individual organisms differ, with these traits representing continuums along which individuals are ordered relative to one another. Definitional aspects of continuity and inheritance make temperament a rich trait with regards to the development of behavior, both in a proximate and ultimate sense. In a proximate sense, the continuity of temperament traits within the individual allows the identification of response styles that can be tracked across the life span. On an ultimate level, the heritability and continuity of these traits provides material for selection over time (Clarke & Boinski, 1995), and allows questions to be asked concerning the development of behavior within a group's ecology and life history. In other words, the study of temperament allows questions such as *how* individuals within a species or group differ from one another, and *why* these tendencies might have developed within a developmental program, or life ecology.

An essential aspect for a population's survival includes the presence of genetically unique individuals which have the ability to exploit non-homogenous resources while responding uniquely to environmental change via new genotypes (Sterrer, 1992). The range of variation seen in the behavior of individuals may be the result of shifting selection pressures, and the maintenance of several types of behavioral responses within a species may allow an animal population to track a varied or changing environment (Danks, 1983; Slater, 1981; Katano, 1987). Thus, temperament traits can be thought of as other types of evolutionary selectable traits (e.g. physical traits), in that the differing levels of expression of these styles provides material upon which selection can occur (Schleidt, 1976; Slater, 1981; Caro & Bateson, 1986; Clarke & Ehlinger, 1987).

Studies that have been performed in nonhuman animals to study psychological traits have included animals from a wide range of taxa: snakes (Herzog & Burghardt, 1988), fish (Francis, 1990; Wilson, et al., 1993), pigs (Forkman, Furuhaug, & Jensen, 1995), wolves (MacDonald, 1983), rats (Garcia-Sevilla, 1984), goats (Lyons, Price, & Moberg, 1988), hyenas (Gosling, 1998), and primates (Watson & Ward, 1996; King & Figueredo, 1997; Byrne & Suomi, 1995; Stevenson-Hinde, Stillwell-Barnes, & Zunz, 1980a; 1980b). For a more comprehensive review of these types of comparative studies, see Gosling (1998) and Gosling & John (1999). Invertebrate individual differences have also been described, although to date only two studies, one with ant workers (Retana & Cerda 1991) and one with octopuses (Mather & Anderson, 1993) have been reported. The latter study provided the first tests for psychological traits in octopuses, which are a logical invertebrate model for the study of behavioral variation due to their intelligence (Wells, 1978) and behavioral complexity (Hanlon &

Messenger, 1996). Mather and Anderson (1993) outlined broad personality traits in adult *Octopus rubescens*, observing three trait dimensions termed *Activity*, *Reactivity*, and *Avoidance*. The current study expands upon this earlier psychological description of octopuses in three ways. First, in an attempt to assess temperament separately from personality and other psychological traits, this study assesses underlying correlations among behaviors that can be identified by the third week of life in *Octopus bimaculoides*. Relatively pure temperamental expression is believed to occur only during infancy or under stressful or novel conditions (Thomas & Chess, 1977; Rothbart, et al., 2000), and measuring young octopuses here as opposed to adults allows for a more specific description of temperament. Secondly, by identifying individuals from known broods and performing measurements at a very young age, this study attempts to assess the influence of relatedness in the expression of relatively innate temperamental traits and contributes to the knowledge of inherited behavioral variation in these cephalopod molluscs. Lastly, this study differs through its assessment of statistical methods used to examine these traits in young *O. bimaculoides*. Choice of statistics, as well as additional statistical methods of assessing factor structure, are discussed and analyzed here.

The Role of Inheritance in Temperament

Due to the difficulty of drawing direct cause and effect relationships between genes and behaviors (Plomin, 1990; Alcock, 1993), the aspect of genetic influence on temperament in this work is worth further note. Genetic influence is thought to play an appreciable, although by no means exclusive role, in the expression of temperament in humans (Buss & Plomin, 1984). However, genetics rarely accounts for more than 50% of the explained variance in expression of these traits in human studies (Plomin, 1990); the remaining variance can be explained through environmental influences (Braungart, Plomin, DeFries, & Fulker, 1992; Thomas & Chess, 1986) and the system of self-assembly of temperament itself (Rothbart, et al., 2000; Thelen, 1990). With this in mind, it is still true that evolutionary change in behavior cannot occur unless there are hereditary differences among individuals that affect their behavior and reproductive success. As a result, demonstrating that genetic differences can be responsible for behavioral differences among individuals is important if we are to have confidence that behavior can evolve (Alcock, 1993).

The study of the heritability of temperamental traits in primates has begun by identifying biological correlates of temperamental trait expression such as hormone levels (Kagan, Resnick, & Snidman 1987, 1988; Kagan & Snidman, 1991). In studies of animals other than primates, genetic influence on behavioral response styles has been shown through testing related individuals while controlling for experiential and situational factors (Herzog & Burghardt, 1988) and also twin studies (Lyons, Price, & Moberg, 1988). Nothing is known concerning the genetic basis of behavior in the cephalopods, but Wells (1958, 1962a, 1962b) postulated that the transition from innately programmed behavioral responses to ones based on learning dictate the early life of young cephalopods and are essential to the young's survival, since from hatching octopuses have no parental care whatsoever (Boletzky, 1987; Hanlon & Messenger, 1996).

The second aspect of this work, then, was to examine whether related *O. bimaculoides* expressed more similar levels of these traits than did unrelated ones. Individual octopuses were tracked by brood, and situational and experiential factors were contolled as much as possible. However, a broad definition of heritability was used here by necessity for a number of reasons. One, individuals in the study could be identified by one parent only and behavioral expression was examined on a phenotypic level. Secondly, due to experimental constraints no controls could be made concerning intra-brood interactions during the first 14 days of life (Appendix A). Still, long-term as well as different developmental contexts were avoided by giving all brood identical rearing conditions, and observations of broods over the first 14 days of life suggested no appreciable differences in behaviors. No studies have been performed to assess heritability of temperament traits in an invertebrate, nor have any accounts been published on the influence of genetic background upon any aspect of cephalopod behavior. Consequently, even with the complexities surrounding this aspect of this study, it is still worthwhile to note differences among broods here in their expression of temperament.

In summary, the aims of this study were: a) to determine whether a set of behavioral observations of 3 weeks old *O. bimaculoides* could be factored into categories reflecting dimensions of temperament; b) to assess the influence of relatedness in individual octopus's expression of these traits; and c) to assess reliability and stability measurements of the factor analysis results.

Materials and Methods

Subjects

Female *O. bimaculoides* with eggs were obtained from the wild (Long Beach, CA area; collected by Chuck Winkler) and shipped to Portland, OR, where all studies were performed. Eight different brooding females were maintained in separate hatching tubs (Appendix C) until eggs were hatched; young animals upon hatching were raised under identical, controlled conditions. At fourteen days of age, individual *O. bimaculoides* were removed from hatching tubs, weighed and measured, and then housed separately in testing containers. The mean wet weight and mantle length for animals at fourteen days old was 1.12 g and 6.53 mm, respectively. Water temperature was maintained at 18 C in all testing and holding containers. Animals were exposed to light according to natural day length (Portland, OR) and fed appropriately sized (size = ½ the mantle length) live shore crabs (*Hemigrapsus*) during the last phase of experimentation on testing days. On days 17 and 18 post-hatching, when testing did not occur, animals were presented with food items (*Hemigrapsus* and *Littorina*) *ad libitum.*

Testing Procedure and Data Analysis

Seventy-three *O. bimaculoides* of known brood type were tested on days 16 and 19 post-hatching. Testing containers were plastic pot holders approximately 10cm in diameter and 10cm deep; the last 12mm of a polystyrene 13 x 100mm culture tube was provided as a den for the hatchlings during these experiments. The top third of the culture tube was kept clear in order to aid observation, the rest of the tube was painted with black epoxy paint. Testing containers were circular, uniform, and opaque on all sides; only their tops were open to permit access for testing. Containers were illuminated with indirect red light; these lights were left on continuously during all phases of experimentation. No experimental studies of daily activity patterns in *O. bimaculoides* have been performed, but personal observations of young animals in the laboratory indicated the hours surrounding dusk as being one of the peak times of activity, thus, tests were begun on each testing day within (+/-) 30 minutes of sunset.

Methods of testing were similar to those used by Mather and Anderson (1993) and were designed to represent 'naturalistic' circumstances. The first test, termed Alert, consisted of the experimenter lifting the opaque lid to the compartment in which the animal was housed in, while leaning over the top of the testing container, effectively casting a shadow over the animal. Octopus behaviors were then recorded for 30 seconds. The second test, termed Threat, occurred directly after Alert, and consisted of the experimenter touching the octopus with an appropriately sized test tube brush (brush diameter = 4mm). Behaviors were again recorded for 30 seconds. The third test, termed Feeding, took place 30-60 minutes after the Threat test. Feeding was a live food presentation (*Hemigrapsus*), and behaviors in this last test were recorded for ten minutes, or until capture of the crab. Instances of behaviors across the three tests for the two different days of testing were then treated as combined frequencies. For recording multiple counts of a given behavior within a test, a 5-second rule was imposed prior to experimentation. This rule counted multiples of a behavior within a test only if the behavior occurred more than once, with a 5 second break in-between occurrences. In other words, to score a '2' for a given behavior, the octopus first had to perform the behavior, then not perform that same behavior for at least 5 seconds, and then perform the behavior again.

Fifteen behaviors (Appendix A) were chosen for analysis from the 19 total observed behaviors. Behaviors were used for analysis if they were contributed at least 5% of the total behaviors expressed during testing. The 15 behaviors considered under this criterion were treated as frequency counts and subjected to exploratory factor analysis (principal components model) with direct oblimin rotation using SPSS 8.0 (Tabachnick & Fidell, 1996). Oblique rotation was chosen on *a priori* grounds that these temperament dimensions were most likely related, and not orthogonal (or uncorrelated) to one another within the psychological make-up of *O. bimaculoides*. Orthogonal rotation was performed as well, and the results matched that obtained by oblique rotation. Since this was an exploratory factor analysis, no *a priori* assumptions were made regarding the number of factors to retain. A number of factors decision was based on: a) a scree test (Cattell, 1966), b) eigenvalues > 1 rule (Kim & Mueller, 1978), c) a parallel analysis (Montanelli & Humphreys, 1976), and d) interpretability of the factors themselves (Zwick & Velicer, 1986). In order to interpret the loadings on each of the factors, behaviors were considered to contribute to the interpretation of a factor if the loading of the behavior on that factor was at least +/-0.4. Acceptable levels of loadings are as low as +/-.32 in some cases (Tabachnick & Fidell, 1996).

Component scores were then computed for each individual on the dimensions that arose from this factor analysis, using a least squares regression method (Tucker, 1971).

Scores were grouped by brood, and means of component scores were computed for each dimension. Component mean scores for broods with a group membership of at least 8 (broods G, X, Y and I; resulting in an N=60) were subjected to ANOVA and Bonferroni post hoc tests (Sokal & Rohlf, 1995) to determine whether or not there were any significant differences by brood in mean factor scores for each dimension.

To assess both experimental as well as statistical methods used here, two additional statistical analyses were performed. The first, a multi-trait multi-method correlation table, was run in order to assess possible experimental or method variance contributing to the results (Campbell & Fiske, 1959). All behaviors from both testing days were grouped by the test in which they were observed (e.g. Alert, Threat, or Feeding) and Pearson correlations were performed among the grouped variables (45 variables total, with a possible 15 behaviors occurring at each of three test situations, resulting in a 45 x 45 correlation matrix). The second additional statistical analysis was a second factor analysis, this time treating the behaviors (variables) as percentages of total behaviors expressed, vs. straightforward frequency counts. The second factor analysis was performed under the same criteria as that used in the original analysis (principal components model, oblique rotation, four factor solution). This analysis was performed to assess stability and structure of the four factor solution obtained through frequency count analysis.

Results

Factor Analysis

Table 1 shows the pattern matrix obtained from principal components analysis of the behaviors recorded during the third week of life of *O. bimaculoides* (n=73). Eigenvalues obtained through this analysis are also given in Table 2 as suggested by Ford, et al., (1986), and were used to assess the number of factors to retain, through the eigenvalue > 1 rule and the scree test. A four factor solution was chosen as the best fitting model of the data, which accounted for 53% of the overall variance in the data.

Naming of each factor or dimension is a subjective process, but involves knowledge about the subjects themselves and should reflect hypotheses concerning underlying causal sources of the groupings of variables (Rummel, 1970). Selection of the factor names here was based on the interpretation of the underlying communalities of the behaviors that loaded highly on a given factor, as well as what those behaviors had in common with one another that was lacking in the behaviors that did not load highly. Thus, names of factors were chosen on the criteria that they reflected the behaviors within each dimension (Appendix B).

The first dimension, named *Active Engagement*, is defined by high loadings (> +/- 0.4) of the behaviors Crawl, Touch Stimulus, Papillae Change, Color Change, Jet, and Arm Probe. The second dimension, *Arousal/Readiness*, is characterized by high loadings of the behaviors Head Move, Respiratory Change, Pupil Change, No reaction (negative loading), and Posture Change (negative loading). A negative loading for a particular behavior can be interpreted as the octopus having a tendency to not display that behavior, in combination with the other behaviors (positive loadings) being displayed. For example, the third dimension *Aggression* can be characterized by octopuses that Grab stimulus, Pull stimulus, and Posture Change (all high positive loadings), while not Jetting from a stimulus (also a high loading, but a negative one). The last dimension, *Avoidance/Disinterest,* is characterized by the behaviors Shrinking, Papillae Change, and not Alerting towards a stimulus (negative loading). Animals can be described along these dimensions as performing the behaviors that loaded highly on each dimension when placed under a novel or stressful situation. For example, an animal with strong *Avoidance/Disinterest* tendencies could be expected to Shrink, perform Papillae Changes, and not Alert towards a stimulus or under novel conditions.

Table 3 shows the inter-factor correlation matrix for the four dimensions found in Table 1. The four factors have low correlations, which suggests that they are unique, separate dimensions in the psychological make-up of *O. bimaculoides*, as the factors were not forced mathematically to be unrelated. The negative sign relationship between *Active Engagement* and *Avoidance/Disinterest* suggests that as an octopus's score increases along the *Active Engagement* continuum, its score decreases along the *Avoidance/Disinterest* one and vice versa. Since all other sign relationships are positive in Table 2, one can characterize individual's scores as increasing along the other continuums relative to one another. Caution is needed here though, since all correlations are below 0.3.

Brood Differences

Table 4 lists the descriptive statistics for the component scores generated through a least squares regression method. A component score was created for each individual on each of the four dimensions for the four broods that had at least 8 members (G, X, Y and I; N=60). Broods with less than 8 members were considered too small for meaningful results in this analysis.

Table 5 shows the ANOVA results, which tested the hypothesis that the variance between broods was significantly greater than the variance within any given brood type for each of the four mean factor scores. In other words, this ANOVA tested whether or not factor scores were significantly different among the four different broods, and provided the analysis of brood differences upon the expression of temperament. Significant differences in factor scores between broods were found for three of the four factors, *Active Engagement* (F=6.542, p<.001), *Arousal/Readiness* (F=9.783, p<.001), and *Aggression* (F=2.933, p<.05). Related individual octopus scored closer to one another along these continuums than did individuals unrelated to one another. There were no significant differences found among any of the four brood types on the fourth dimension, *Avoidance/Disinterest.*

Characterizations of the broods along the four dimensions can be achieved through examination of component mean scores (Fig. 1). Thus, G brood members could be described as displaying median amounts of *Active Engagement*, high levels of *Arousal/Readiness* (significantly higher than X brood, p<.001), and high levels of both *Aggression* and *Avoidance/Disinterest* behaviors. G individuals had the highest average among broods tested in the expression of *Arousal/Readiness* and *Aggressive* behaviors, although their scores were not significantly different from Y brood along this latter dimension, which can be attributed to these two groups' sample sizes. G group members were extremely *Reactive*, *Aroused*, and *Aggressive*, but were not particularly *Active* or *Interested* in seeking out contexts in which to be so.

X brood members can be described as highly *Actively Engaged* (significantly higher than I brood, p<.001), not at all *Aroused* or *Ready* (significantly lower than I brood, p<.05 and G brood, p<.001), highly *Aggressive* (significantly higher than Y, p<.05), and not very *Avoidant* or *Disinterested.* X brood also represented the group that was the most different from the other three, scoring significantly different from the other groups on three of the four dimensions. This brood at a very early age was particularly *Active* in *Engaging* with their environment, acting *Aggressively*, effectively choosing to act towards or approach stimulus as opposed to assessing situations from afar, or *Avoiding* them altogether.

Y brood members are described here relative to the other groups in the study as mildly *Actively Engaged*, mildly *Aroused* or *Ready*, not *Aggressive* (significantly lower than X brood, p<.05) and mildly *Avoidant/Disinterested.* Y brood seems to represent a modal group, or one that was not at any extremes, although it did score the lowest along the *Aggression* dimension.

I brood members were the lowest in scores along *Active Engagement* (significantly lower than X brood, p<.001), while being highly *Aroused* and *Ready* (significantly higher than X brood, p<.05), displaying somewhat high levels of *Aggressiveness*, and mildly *Avoidant* or *Disinterested.* Being not very *Active* or *Engaged* in their environment, but highly *Aroused* and *Aggressive*, and mildly *Avoidant* would describe this brood of octopuses.

Examination of Test Validity

Two additional statistical tests were run to assess the validity of the findings as well as the stability of the factor structure. The first, a multi-trait multi-method test, analyzed whether the factors that arose in the four factor solution were true indicators of octopus temperament or were constructs that arose due to testing methods. The second was a second factor analysis which examined whether treating variables as percentages rather than frequencies would affect the factor structure outcome.

The multi-trait multi-method (MTMM) correlation table is not shown here, due to size limitations (45 x 45 correlation matrix, available from author). Under investigation in this correlation table was whether or not there was test or method variance contributing to the final pattern matrix solution, or temperament dimension interpretation. If significant correlations among behaviors within a test outweighed significant correlations within behaviors across tests, it could then be argued that a dimension arose due to testing methods, and not necessarily due to its presence in the psychology of the octopuses tested (Cambell & Fiske, 1959). Among correlations between behaviors within each of the three test situations, 294 correlations were examined, and resulted in 38 significant correlations at the $p<.05$ level. Within the 15 behaviors in each test scenario (45 total correlations), 9 significant correlations were found. The proportion of these two validity measurements is 0.13 and 0.2, respectively, indicating that there was a higher proportion of convergence within behaviors than method variance. Furthermore, no discernable pattern of significant correlations was observed, either within behaviors or among behaviors within a given test. These results indicate that behaviors were stronger predictors of themselves than of any given test situation, and suggest construct validity for the temperament dimensions that arose under this test scenario.

Table 6 is the pattern matrix obtained from the second factor analysis which examined the stability of the four factor solution. In this second analysis, behaviors were treated as percentages of total behaviors observed rather than frequency counts. This increased variance among variables, and factor analysis of these variables allowed the assessment of a different variable form upon the factor solution. The results that were obtained matched that of the frequency variables, illustrating the stability of the factor solution derived from using frequency counts. Due to its clarity in method as well as the patterns of loadings obtained, treating the behaviors as frequency counts was determined to be the most accurate in describing the data and was used for all further analysis.

Discussion

The development of intelligence in octopuses through evolutionary time has been proposed to have been a result of the complexity of the near shore reef environment as well as through their competition with the bony fishes (Packard, 1972; 1988b; Mather, 1995). The study of the psychology of the octopuses also informs us about this development of behavior through time as well, since temperament is thought to function as a dynamic moderator variable between behavioral expression of the individual and the context in which it is expressed (Mason, 1984). This analysis suggests that *Octopus bimaculoides* show individual differences in behavior, or temperament, on at least four different dimensions (*Active Engagement*, *Arousal/Readiness*, *Aggression*, and *Avoidance/Disinterest*) at 3 weeks of life, and that these behavioral traits are expressed differently depending on brood of the individual.

Temperament Factors and Life History of Octopuses

In an ultimate sense, how does temperament relate to the behavior of octopuses? Clark & Ehlinger (1987) have suggested that heterogeneous environments are a major selective pressure towards the development of individual differences in animals. Certainly near shore octopuses are subjected to development under variable contexts. They are known to inhabit wide variety of fluctuating environments including sand, rock, and mud bottoms (Lang, 1997), as well as reef settings with variable prey and predator densities and habitat choices (Ambrose, 1988). Octopuses in these environments display variable, irregular behavior patterns, such as prey selection (Ambrose, 1984; Mather, 1991), den site selection (Mather, 1994) and movement patterns (Ambrose, 1988; Mather & O'Dor, 1991). This complexity of their environment coupled with their competition with the fishes has dictated that the soft-bodied cephalopods develop complex behavior repertoires (Robinson, 1990; Mather, 1995). Temperament in octopuses may provide an initial, innate basis for the development of these complex behavior patterns that allows them to survive under fluctuating selective pressures.

On a proximate level, the behavioral style of *Active Engagement* suggests that young octopuses differ on the amounts that they move and explore their environment. This dimension may be important as we consider how an individual integrates its experience in order to maximize energy gain and ensure survival. Under certain circumstances, such as low predator density, it might be adaptive for young octopuses, such as X brood members, to be highly *Actively Engaged* in their environment, maximizing prey interaction and uptake. Conversely, under a high predator density context, these highly *Actively Engaged* animals may not have the same likelihood of continued success, while octopuses that display lower amounts of these traits (I brood members) could maximize opportunistic circumstances while avoiding predation. Indeed, Mather and O'Dor (1991) described juvenile *O. vulgaris* in Bermuda as exploratory and opportunistic, but inactive and postulated that this observed strategy was influenced by predation pressure on the young.

Similarly, differences along *Arousal/Readiness* trait levels can be interpreted as differences in levels of expression of behaviors that include assessment from afar, without actually engaging with a stimulus. An assessment of an object without coming in contact with it could protect young animals from potentially harmful interactions with previously unencountered predators, and would be important in an octopus's ability to learn from a distance while also maintaining survival. Animals with high levels of *Arousal/Readiness* responses, such as G brood members, may increase chances for survival in a high predator density context by assessing novel stimuli first before actually interacting with that stimulus. This dimension could also play a role in the internal build up preceding 'fight' or 'flight' reactions, which has been a suggested role for arousal traits in humans (Buss & Plomin, 1984).

Aggressive behaviors shown by young *O. bimaculoides* represent a wide-ranging behavioral style that holds importance for many different groups of animals (Gosling & John, 1999). Selection for variability along *Aggressive* traits has been documented by Smith & Harper (1988), who described 3 separate models in which natural selection could maintain genetic variability for *Aggression* in midas cichlids and songbirds. Similarly, other cephalopod behaviors such as characteristic color patterns have been assigned as indicators of aggressiveness (in *Sepia* - Corner & Moore, 1980; Adamo & Hanlon, 1996). For young octopuses, size dependent interactions may require differing levels of expression of this trait within an individual, since attacking a potentially dangerous food item larger than oneself would not be a particularly well-suited survival strategy. Yet, *Aggressive* expressions of behavior may be of benefit to these young animals as they come in contact with similar sized octopuses as well as other competitors for food and/or shelter resources (Caldwell & Lamp, 1981; Mather, 1982). Lorenz (1963) postulated that one of the results of intra-specific aggression is the spatial distribution of a species, and he considered *Aggression* to be one of the basic drives of organisms along with feeding, reproduction and flight.

How might *Avoidance/Disinterest* be of importance to young octopuses? Possibly, this trait encompasses another route by which a young individual may learn from its environment (Mather & O'Dor, 1991) while not being preyed upon. Foraging strategies of animals may emphasize *Avoidance* of predation (Lima & Dill, 1990), and if octopuses do indeed adopt this strategy in the wild, this dimension would represent the behaviors upon which initial levels of these strategies could be used. Individual *O. bimaculoides* tended to act more similarly along this trait than any other since there were no significant differences found among the broods. Whether or not this trait possesses significant behavioral variation among the octopuses remains to be seen.

Heritability of Temperament in *O. bimaculoides*

One of the major postulated influences on temperament is the role of inheritance (Plomin, 1981). The development of behavior response styles through genetic expression is highly complex (Thelen, 1990; Oyama, 1985), and most likely involves a self-regulating, nonlinear, dynamic system that interacts both within itself and with the external environment (Thelen, 1990). Therefore, a simple relationship such as "genes make traits" does not exist. However, the possession of a particular kind of genetic information does have biochemical consequences for the development of individual differences, and it is for this reason that genetic influence in temperament response styles was further examined as part of this study.

Studies with animals other than primates have begun to demonstrate this relationship (garter snakes - Herzog & Burghardt (1988), Arnold & Bennett (1984); dairy goats - Lyons, Price, & Moberg (1988); pigs - Forkman, Furhaug, & Jensen, (1995)), but little is known concerning the genetic basis of behavior in cephalopods. Wells (1958; 1962a; 1962b) postulated that the cephalopods must be born with a behavioral repertoire based on innate mechanisms, and temperament theory suggests that these early innate expressions of behavior are based on temperamental aspects of the organism (Goldsmith, et al., 1987).

In order to determine the role of genetic influence on temperament response styles in *O. bimaculoides,* experiential and situational factors were minimized by testing very young octopuses, providing identical raising environments, and giving subjects the same tests. One possible confounding factor is that while broods were always separated, octopuses within broods had to be raised together for the first two weeks prior to individual separation. However, even with the limits of my experimental controls, the observed brood differences in temperamental trait expression would suggest the potential of a detectable genetic component to these traits. Further investigation will be needed to delineate this genetic component.

Descriptions of brood patterns along the four dimensions may indicate life history strategies used by different animals within this species. Developmental theorists describe those animals on the extreme ends of temperamental traits as having the greatest effects on the environment, and those that fall into the middle of the continuum of traits such as these as being most affected by their environment (Buss & Plomin, 1984). How might an extreme group, such as the X brood individuals, affect their environment differently than a more modal group, such as Y brood? Trophically, octopuses can be top level predators (Mangold, 1983; Ambrose, 1988), being a major influence on the abundance of organisms beneath them in the food web. It may be that these more extreme individuals under certain circumstances shape the profile of these trophic interactions more radically within their environment, while a more modal group would simply function as one of the levels within the trophic web, not influencing its profile as heavily as extreme individuals. Octopuses such as G brood individuals might maximize their energy intake with their tendency towards highly *Aggressive* and highly *Aroused/Ready* responses, while ones more similar to I brood members may represent the emphasis of survival over energy maximization through low *Active Engagement* and high *Avoidance/Disinterest* behaviors. The individual/environment interaction and development is a currently untouched area of research for octopus, but would undoubtedly be a rich area of study.

Since the eggs of wild caught brooding females were used in this study, no controls were able to be made as to the father of each individual. This aspect also contributes to the broad definition of genetic influence used here, since only mothers could be identified. Hanlon (1983) observed multiple male *O. briareus* mate with the same female in the wild, and adult *O. bimaculoides* have been observed mating with more than one partner in the laboratory (Forsythe & Hanlon, 1988a; personal observations). Consequently, the likelihood of more than two parental types contributing to the results here is very real, making assumptions concerning the relatedness of siblings more difficult. Furthermore, the greater similarity in scores within broods than across broods could be attributable to intra-brood interactions during the first 14 days of life. However, daily 30 min. observations of individuals during this initial two weeks suggested that no broods differed significantly in their interactive activity, and similar individual spacing was observed during these initial conditions. Even though differences among broods cannot, at this point, be completely attributed to genetic background of individual octopuses, they suggest it will be worthwhile to examine the influences of heritability on the behavior of individual octopuses, and the results here will contribute to these types of study in *Octopus*.

Additional Assessment of Experiment and Statistical methods

Due to the number of choices researchers face with regard to factor analytic studies, such as rotation method and number of factors to retain (Tabachnick & Fidell, 1996), additional hypothetical and statistical steps were taken here. These were performed in order to ensure construct validity as well as to illustrate methods available to animal behaviorists interested in conducting similar studies with their subject organisms. First, oblique rotation was chosen here vs. an orthogonal method, which is a more commonly used rotation. This was done in order to better represent the complexity of the examined behaviors, which are most likely uncorrelated (Harman, 1976). Oblique rotation does not force factors to be unrelated to one another mathematically, thus paralleling the physical structure of the dimensions within the organism as well. Orthogonal rotation assumes that psychological factors are wholly unrelated to one another, a scenario which does not seem likely given the complexity of individual systems (Thelen, 1990). While most researchers prefer orthogonal rotation due to ease of interpretation (Gosling, pers. comm.), it seems worthwhile for animal behavior researchers to assess the underlying mathematical assumptions surrounding this method in relation to their subject organisms when making choices concerning method of rotation.

As suggested by Ford, et al., (1986), who noted the disparity of information reported for factor analytic studies in the psychological literature, further detailed results were given here by reporting the basis for the number of factors decision (Methods), as well as presentation of the pattern matrix (Table 1) and eigenvalues of vectors (Table 2) obtained through principal components analysis. By reporting these values, other researchers have an opportunity to objectively assess the interpretation of factor analytic results, as well as allowing for increased replicability of results.

Lastly, two additional statistical tests were used to assess the validity and stability of factor structure. The first, a multi-trait multi-method analysis, examined possible test variance contributing to the results. A second factor analysis examined the role of variable form upon the factor solution. Examining separate factor analytic results of the same data using differing types of variables can give valuable information regarding a factor solution. If a factor solution arises due to a mathematical constraint, such as low variance due to the type of variable used (e.g. categorical variables), examining the factor solution derived from the same variables in a different form (e.g. continuous variables) may point out the true factor solution. Assessment of test methods through multi-trait multi-method analysis may be worthwhile to researchers as well who have used different types of tests and are interested in the construct validity of their findings. This type of analysis seems especially relevant when using questionnaire type data, where researchers develop their own questions specifically to target particular constructs.

Chapter Conclusions

Behavioral polymorphism in offspring has been identified as an evolutionarily stable strategy that maximizes survival of a species during fluctuations in social and ecological conditions (Bekoff, 1977). Frequency dependent selection could also make phenotypic deviation from the norm advantageous (Clark & Ehlinger, 1987). Recently, the nature of individual differences in populations has been reported (Colgan, Gotceitas, & Frame, 1991; Francis, 1990; Rosenzweig, 1991), and a fundamental shift seems to be occurring as our thinking changes from the level of the averaged behavior in the population to the level of individual differences (Wilson, et al., 1993; Stevenson-Hinde & Zunz, 1978).

It is clear that there are correlations among behaviors of young *Octopus bimaculoides* that can be called temperament and these traits may be influenced by the relatedness of individuals. Applying the concept of temperament to octopuses informs us about the behavioral polymorphism of the young of this group, as well as providing a proximal bases with which to study inter-specific and intra-specific variation in behavior. These variations in the young may point to diet and habitat specializations and would provide the initial levels of behavioral expression for a young octopus. The role of temperament and its relation to the behavior and life history of octopuses is an important aspect of the psychology of this group that is in need of further study.

Table 1 – Factor loadings of the frequency count behaviors displayed by 3 week old *O. bimaculoides* on four direct-oblimin rotated principal components, n=73

	Active Engagement	Arousal/ *Readiness*	Aggression	*Avoidance/ Disinterest*
Touch Stimulus	**.716**	-.096	-.067	.041
Crawl	**.815**	.069	-.225	-.075
Papillae Change	**.649**	.284	-.036	**.442**
Color Change	**.715**	-.060	.242	-.170
Jet or Swim	**.544**	.188	**-.466**	-.155
Arm Probe	**.558**	-.271	.138	-.001
No reaction	-.351	**-.553**	-.353	.346
Head Move	.018	**.460**	.061	.013
Respiratory Change	-.022	**.615**	.190	.112
Pupillary Change	-.115	**.731**	-.095	-.170
Posture Change	.173	**-.498**	**.440**	-.321
Grab Brush	-.092	.227	**.664**	.058
Pull Brush	.012	.043	**.626**	.100
Shrink	-.067	-.030	.082	**.693**
Alert Posture	-.059	.057	-.073	**-.707**
% Variance Explained	20.3	13.3	10.2	9.2
% Variance (Total)	53.0			

Note: The highest factor loading(s) for each behavior is listed in boldface type.

Table 2 - First ten eigenvalues obtained through principal components analysis of frequencies of fifteen behaviors performed by *O. bimaculoides* (n=73) during testing in its third week of life.

Component	*Eigenvalue*
1	3.046
2	1.992
3	1.532
4	1.384
5	1.130
6	1.002
7	.933
8	.797
9	.735
10	.632

Table 3 - Inter-factor correlation matrix of the four temperament dimensions for *O. bimaculoides* at 3 weeks of age (n=73)

Component	*Active Engagement*	*Arousal/ Readiness*	*Aggression*	*Avoidance/ Disinterest*
Active Engagement	1.000			
Arousal/ Readiness	.015	1.000		
Aggression	.016	.021	1.000	
Avoidance/ Disinterest	-.142	.071	.027	1.000

Table 4 - Descriptive statistics of component scores for members of four broods of *O. bimaculoides* (n=60) along their four dimensions of temperament

		N	**Mean**	**Standard Deviation**
Active Engagement	G brood	16	-.2806	.4358
	X brood	25	.1316	.7015
	Y brood	11	-.3440	.4597
	I brood	8	-.8387	.4356
	total	60	-.1949	.6436
Arousal/ Readiness	G		.7181	1.1298
	X		-.6984	.7808
	Y		.0044	.5632
	I		.4204	.8058
	total		-.0426	1.035
Aggression	G		.3634	.8782
	X		.3293	.9668
	Y		-.5975	.9509
	I		.1566	.9126
	total		.1455	.9790
Avoidance/ Disinterest	G		.4357	.9049
	X		-.2404	.9793
	Y		.0985	.8086
	I		.1978	.9910
	total		.0605	.9513

Table 5 - Results of ANOVA testing four different brood types on the four dimensions of temperament (n=60)

		Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Active Engagement	Between Within Total	6.343 18.100 24.443	3 56 59	2.114 .323	6.542	.001
Arousal/ Readiness	Between Within Total	21.748 41.496 63.244	3 56 59	7.249 .741	9.783	<.001
Aggression	Between Within Total	7.679 48.873 56.552	3 56 59	2.560 .873	2.933	.041
Avoidance/ Disinterest	Between Within Total	4.682 48.710 53.393	3 56 59	1.561 .870	1.794	.159

Figure 1 - Component mean scores of octopus broods on four dimensions of temperament, n=60

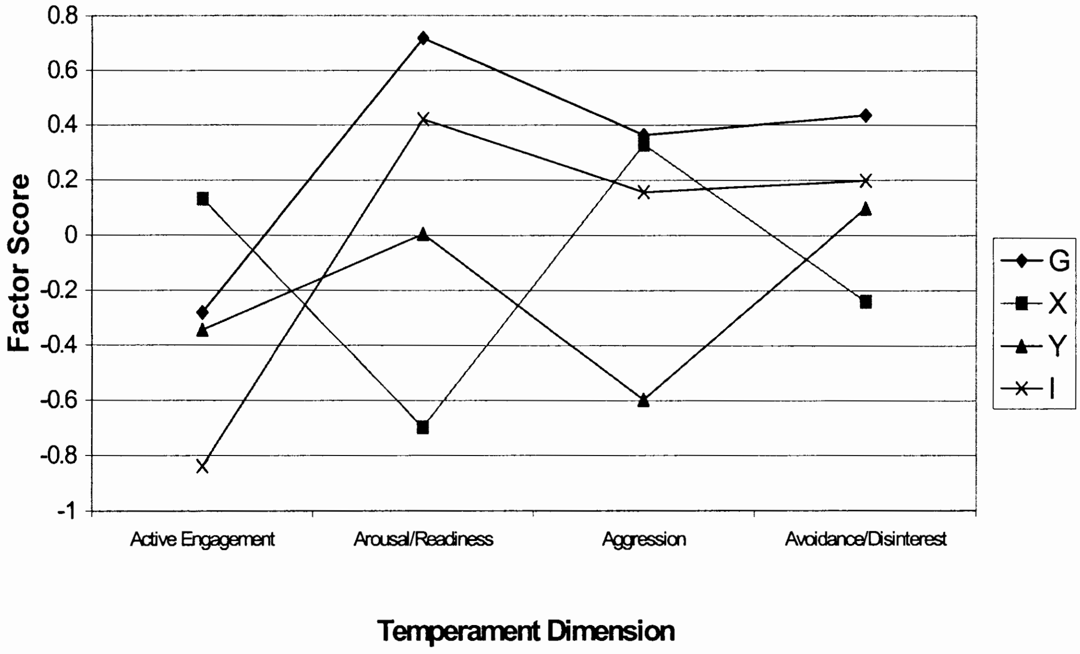


Table 6 – Factor loadings of the behaviors displayed by 3 week old *O. bimaculoides* on four direct-oblimin rotated principal components, n=73; Behaviors expressed as percentages of total behaviors observed

	Active Engagement	Arousal/ *Readiness*	Aggression	*Avoidance/ Disinterest*
Touch Stimulus	.013	.019	-.308	**-.528**
Crawl	**-.647**	-.181	.379	-.287
Papillae Change	.082	**.649**	-.270	-.190
Color Change	-.004	-.162	.013	**-.596**
Jet or Swim	**-.698**	.065	.104	-.063
Arm Probe	.009	-.320	-.037	-.223
No reaction	.004	-.354	-.233	**.812**
Head Move	-.125	.271	-.367	.176
Respiratory Change	.118	**.711**	-.144	-.133
Pupillary Change	.009	**.619**	.266	.063
Posture Change	**.391**	**-.460**	-.341	-.271
Grab Brush	**.588**	.040	**.443**	.216
Pull Brush	**.596**	.167	.328	-.116
Shrink	.245	.009	-.130	**.805**
Alert Posture	-.043	-.001	**.679**	-.028
% Variance Explained	10.6	12.2	9.8	18.1
% Variance (Total)	50.7			

Note: The highest factor loading(s) for each behavior is listed in boldface type.

Chapter 2 - The development of temperament in *Octopus bimaculoides*: a behavioral assessment over the first nine weeks of life

Introduction

Temperament is defined as individual behavioral tendencies that display continuity over time, can be identified in early infancy, and have a genetic basis (Buss & Plomin, 1984; Fox, 1989; Healy, 1989; Kagan & Snidman, 1991). Aspects of heritability are thought to contribute to the relative stability of temperamental expression across time and in differing situations (Buss & Plomin, 1984), as well as allowing for the adaptive selection of these behavioral styles in organisms (Clarke & Boinski, 1995). Developmental researchers have utilized longitudinal research designs on their subjects in order to examine the basis of formation and development of temperament over time in their subject organisms (Thomas & Chess, 1977; Buss & Plomin, 1984; Plomin & Dunn, 1986), with studies concerning humans (Kagan, 1971; Moss & Sussman, 1980; Mednick, Harway, & Finello, 1984) and other nonhuman primates (Stevenson-Hinde et al., 1980a; 1980b) providing the earliest studies on the development of temperament traits. Recently, however, other taxa have been included in these types of studies in a comparative attempt to understand the formation and development of these trait styles. Goats (Lyons, et al., 1988), cichlid fishes (Francis, 1990), wolves (MacDonald, 1983), snakes (Herzog & Burghardt, 1988), and gerbils (Cheal & Foley, 1985) have provided us with comparative knowledge of the development of temperament among the vertebrates, while a longitudinal study of individual differences among *Cataglyphis* *cursor* ant workers (Retana & Cerda, 1991) has provided insight into the development of an invertebrate's individual variability.

Developmental phenomena can be thought of as being characterized by both continuity and change, since development by definition involves some degree of change over time, but not every aspect or feature of an organism necessarily changes throughout the process of development (Suomi, Novak, & Well, 1996). Both constancy and change are major developmental paradigms reported from studies examining early personality traits, or temperament, in vertebrates (Suomi, et al., 1996; Stevenson-Hinde, et al., 1980a, 1980b; Lyons, et al. 1988; MacDonald, 1983). Developmental studies of individual differences present a paradox, then, since change is an essential aspect of any developmental function, and yet some element of stability or consistency is also necessary for the maintenance of individual distinctiveness (Sackett, Sameroff, Cairns, & Suomi, 1981). Indeed, temperament is defined by the stability of its components over time (Goldsmith, et al., 1987).

The study of individual differences among octopuses provides a comparative view of the development of individual variance in a highly divergent organism from the vertebrates previously studied for these traits (Mather & Anderson, 1993; Mather, 1995). Previous studies with octopuses have included the examination of broad personality traits in adult *Octopus rubescens* (Mather & Anderson, 1993) and the definition of early temperamental traits in *Octopus bimaculoides* (Chapter 1). This work characterizes both continuity and change in the development of temperament across the first nine weeks of life in *O. bimaculoides*.

Three approaches were used to describe the development of individual differences in *O. bimaculoides,* similar in method to Higley, Suomi, & Linnoila (1990) and Suomi, et al., (1996). The first approach describes the discrete behavioral frequencies performed at each testing week, and analyzes change at the level of discrete behaviors through repeated-measures ANOVA (Tabachnick & Fidell, 1996). Observed behaviors are the measurement variables used throughout this study, and are also the variables upon which octopus temperament is based (Chapter 1). Discrete behavioral studies are also the traditional means for studying the development of behavior among the octopuses as well (Wells & Wells, 1970; Mather, 1984). Thus, discrete behaviors are the traditional variables upon which behavioral development is based.

However, the second and third approaches in this study attempt to illustrate the advantages of examining the development of octopuses at a level of broader dimensions of behaviors, or temperament. The second approach is a normative one, and creates a developmental function description for the group of animals tested (Chalmers, 1987; Wohlwill, 1973). This approach compares the patterns of composite scores generated from the four temperament dimensions outlined in Chapter 1, at week three, week six, and week nine post-hatching. It examines the relative stability of these patterns over time, or, in other words, asks whether or not scores on the four temperament dimensions for *O. bimaculoides* vary relative to one another depending on the age of the animal. Which developmental patterns are different from one another (week 3, week 6, or week 9) and where these differences arise (the particular temperament dimension) are also the subjects of this second analysis.

The third approach describes the differences and similarities in development of behavioral styles of octopuses grouped by relatedness, or brood. This analysis tests whether individuals that are related to one another develop differently from those that are not, and assesses continuity and change from a level of brood type. This is important since the expression of temperamental traits as a function of relatedness has rarely been demonstrated outside of the primates (but see garter snakes - Herzog & Burghardt, 1988; goats - Lyons, et al., 1988; and octopuses - Chapter 1).

These differing statistical approaches provide different windows on developmental continuity and change in *O. bimaculoides* and yield distinct yet complimentary views of the same phenomenon. The consistency and compatibility of the approaches with one another are examined here along with the extent to which using multiple methods to describe the same developmental data provides additional insight beyond any single technique. Analysis of broader dimensions of behavior indicates both change and continuity in these organisms, and allows hypotheses to be generated concerning underlying developmental processes, mechanisms, and influences in the early life history of *O. bimaculoides*. In contrast to this is the view that arises from the analysis of discrete measurements by themselves, which do not indicate these changes and may not be as informative. Temperamental trait development is characterized by constancy and change, and should be influenced by the genetic background of the individual (Buss & Poley, 1976; Buss & Plomin, 1984). Since we know so little about temperamental trait development in any invertebrate and since their life history is so different from vertebrates previously studied for these traits, *Octopus* provides an excellent model in which to study the development of temperament.

Methods

Subjects

Thirty-seven *O. bimaculoides* of known age and brood were given behavioral tests during their first nine weeks of life to assess both discrete behavior and temperamental dimension development. Methods of feeding and testing were described in Chapter 1 and were followed for all subsequent testing weeks. Animals were housed in separate testing containers for all phases of testing and once they were placed in these separate containers were not moved. For a description of testing containers, see Chapter 1. Three behavioral tests described in Mather & Anderson (1993) and also described in Chapter 1 were used during all observations, and consisted of an Alert test, a Threat test, and a Feeding test. These three tests were conducted twice per week during week 3, week 6, and week 9 post-hatching, with at least a two-day break between the set of tests within each week. Behaviors used for analysis were those that made up at least 5% of the total behavioral responses in tests given at week 3 (Appendix A).

Data Analysis

In the first analysis approach, behavioral frequencies observed during each week were summed across both testing days for each individual. These frequencies were then summed for the group by week and averaged, resulting in a single frequency average for each of the fifteen behaviors for each testing week. These within behavior frequency averages were subjected to repeated measures ANOVA in order to determine which behaviors changed significantly across the three time points. When significant differences were found for a given behavior, both simple and deviation contrasts were used to determine which pairings of frequencies within that behavior were significantly different from one another (2 pairs possible: week 3 vs. week 6 frequencies, and week 6 vs. week 9 frequencies).

For the next two analysis approaches, composite scores were generated for each individual for each temperament dimension at each time point, by using a method similar to that of Stevenson-Hinde, et al. (1980a). In Chapter 1 methods were given in regards to the principal components analysis at week 3, which resulted in four factors, *Active Engagement*, *Arousal/Readiness*, *Aggression*, and *Avoidance/Disinterest*. The pattern matrix is given in Chapter 1, Table 1, and it is this pattern matrix upon which composite scores were based. In order to generate a composite score along a temperament dimension for an individual, variables that loaded 0.4 or above on each factor in Chapter 1, Table 1 were summed for that particular time point. For example, a composite score at week 3 was created for *Active Engagement* by summing the frequencies observed during that week for the behaviors Crawl, Touch Stimulus, Papillae Change, Color Change, Jet, and Arm Probe. Similarly, a composite score at week 3 was also created for *Arousal/Readiness* by summing the frequencies observed during that week for the behaviors Head Move, Respiratory Change, and Pupil Change, while subtracting frequencies observed for No Reaction and Posture Change, which were both negative loadings on this factor. In this way four composite scores for each individual were created at each time point, resulting in three sets of behavioral scores which allowed analysis of the development of broad dimensions of temperament.

These composite scores were then used to create a developmental function for the entire group of 37 individuals. Composite scores were averaged across all individuals for each of the four dimensions at each time point, giving a single mean score on *Active Engagement*, *Arousal/Readiness*, *Aggression*, and *Avoidance/Disinterest* at each of the three testing weeks. Profile analysis was then used to assess parallelism of the behavior patterns across the three different time points (Tabachnick & Fidell, 1996). Parallelism tests the hypothesis that there is no difference in the patterns of behavior scores at week 3, week 6, and week 9. A significant interaction for this analysis indicates that a given pattern of scores is different across time points. Significant differences were further examined through simple and deviation contrasts, which tested scores on each temperament dimension separately at the three different time points.

A third analysis approach was used to describe the development of individual octopuses by brood, and allowed for assessment of brood influence on the expression of temperament traits. Profile analysis was again used to assess differences in parallelism of behavior patterns, but in this approach among selected pairs of broods as opposed to three separate time points. Three different pairs of broods were tested, X with Y, X with I, and Y with I. Profiles of behavior patterns were produced for each brood across all three time points, and significant differences were examined through simple main effects testing (Tabachnick & Fidell, 1996).

Results

Figure 2 displays the average frequency of the fifteen discrete behaviors averaged over all individuals at each time point. Significant changes in mean frequencies over time were found for three of the fifteen behaviors: Crawl (F=6.206, df=2, p<.05), Respiratory Change (F=12.470, df=2, p<.001), and Posture Change (F=4.023, df=2, p=<.05). Simple and deviation contrasts were used to examine the pairings of time points within these three significant main effects. Crawl frequencies decreased from week 3 to week 6, but not significantly, while the decrease from week 6 to week 9 was significant (F=20.233, df=1, p<.001). Respiratory Change frequency differences were significantly higher at week 6 than week 3 (F=18.641, df=1, p<.001) but non-significant differences were seen in the increase in average frequencies from week 6 to week 9. A significant decrease in mean frequency of Posture Change was observed from week 3 to week 6 (F=5.495, df=1, p<.05) with a non-significant increase in frequencies from week 6 to week 9. Since only 3 out of 15 behaviors tested changed significantly in this analysis, this approach was interpreted as yielding a view of consistency in the expression of behavior across the first nine weeks of life of *O. bimaculoides*.

The developmental function pattern of the four temperament factors across the three time points for all animals is shown in Figure 3. An assessment of parallelism revealed a significant difference between patterns for the three weeks tested (F=3.283, df=6, p<.05). This significant difference among composite scores can be seen between weeks 3 and 6 (Fig. 3). *Arousal/Readiness* scores are significantly lower at week 3 than at week 6 (F=6.7, df=1, p<.05), while *Aggression* scores are significantly higher at week 3 than at week 6 (F=6.529, df=1, p<.05). A decrease in *Active Engagement* score from week 3 to week 6 was also observed, although this change was non-significant. The change in composite score from week 3 to week 6 for the fourth dimension, *Avoidance/Disinterest*, was minimal and was not significant either. Week 6 was not significantly different than week 9 on any of the four behavioral composite scores.

The third approach compared patterns of temperament development across different broods of octopuses. Significant differences in patterns of this behavioral development across the three time points were found between Y and I broods (F=4.51, df=6, p<.001) as well as Y and X broods (F=4.21, df=6, p<.001), indicating that the behavioral dimensions of temperament in *O. bimaculoides* develop differently depending on the brood of the individual. A non-significant difference was found in the third pairing of broods, X and I broods.

The broad patterns of behaviors for the brood types can be described qualitatively, and can be used to characterize behavioral profiles among different broods of octopuses. At week 3 Y brood is seen to have divergent scores among *Active Engagement* and *Arousal/Readiness* dimensions (significantly different from I brood, F=6.779, df=3, p<.001; see Fig. 5) that over time converge with one another (Fig. 4) resulting in a significant difference from brood I at week 9 (F=3.561, df=3, p<.05). Week 6 was not different for the pairing between I and Y broods. Among the four dimensions of temperament, the pattern of the *Arousal/Readiness* scores was found to differ significantly between these broods (F=11.86, df=2, p<.001). I brood exhibits the opposite pattern from Y brood along these first two dimensions, with composite scores for *Active Engagement* and *Arousal/Readiness* that are close to one another at week 3 while diverging relative to one another through the testing period (Fig. 5). *Aggression* scores also decrease from week 3 to week 6 while increasing in week 9, with *Avoidance/Disinterest* scores also showing a fluctuating pattern across the nine weeks. The pattern of composite scores exhibited by X brood is variable, with scores along the *Active Engagement*, *Arousal/Readiness* and *Aggression* dimensions fluctuating across the nine weeks and *Avoidance/Disinterest* scores remaining relatively stable (Fig. 6). Between Y and X broods, significant differences were found in the patterns of the four composite scores at week 3 (F=7.690, df=3, p<.001) but not week 6 or week 9. Again, only *Arousal/Readiness* was found to have a significantly different pattern (F=8.007, df=2, p<.001) between these two broods on four dimensions of temperament. No significant differences were found in the comparison of the patterns of development between I and X broods, nor among composite scores.

All three approaches gives a slightly different view of development among octopuses, yet the patterns that arise from each can contribute to an overall view of constancy and change. While discrete behaviors indicate little change, the normative developmental pattern as well as the patterns of development taken at the brood level may be better indicators of behavioral change in octopuses. All three approaches indicate weeks 3 to weeks 6 as being the time of greatest change, while the second and third approaches indicate that these changes are taking place among the dimensions *Active Engagement*, *Arousal/Readiness*, and *Aggression*. *Arousal/Readiness* accounts for the greatest variance between broods of octopuses tested here.

Discussion

Among the octopods, there is a scarcity in the literature concerning systematic studies of the development of behavior (Hanlon & Messenger, 1996). However, Mather (1984) pointed out the importance of such studies as they apply to the life history of octopus, both in terms of outlining ethological descriptions and in terms of learning about octopus adaptation to their environment. This study here is the first description of the development of behavioral style in this group of animals, as well as providing the first systematic study on the development of discrete behaviors in *O. bimaculoides*.

Octopus behavioral development

How can the data here be applied to our knowledge of the life history of these organisms? In primates, ecological specializations have been associated with temperament differences, with studies showing that species that depend on manipulative and explorative foraging techniques tend to be more highly inquisitive and less reactive to novelty than other species (Clarke & Lindburg, 1993; Vitale, Visaberghi, & DeLillo, 1991). Chapter 1 discusses possible adaptive reasons for patterns of dimension scores at week 3 for *O. bimaculoides*, but how might the overall pattern of development of these dimensions for the group be linked to an ecological context as well?

While examination of development at the level of discrete behaviors may not be as informative of change in the early life of octopuses (12 out of 15 behaviors indicate no significant changes), examination of the broader dimensions of temperament may be more informative of these changes while also allowing generalizations to be made concerning ecological correlates of behavior patterns. Animals can be described at week 3 relative to week 6 and week 9 as being more *Actively Engaged* in their environment, not very *Aroused* or *Ready*, more *Aggressive*, and equally *Avoidant* or *Disinterested.* How might it be important for these animals at week 3 to be more highly mobile, while also attacking or being more aggressive in their initial responses to stimulus? *O. bimaculoides* immediately upon hatching assumes a benthonic existence, but may have a swimming stage upon hatching for dispersal purposes (Lang, 1997). This early tendency towards movement has been postulated to serve dispersal purposes in other octopods as well (*O. joubini* - Mather, 1984). Similarly, not being as *Aroused* when presented with novel objects at this earlier time could complement tendencies towards *Active* and *Aggressive* actions, since *Arousal/Readiness* measures do not include spatial movements. This increased tendency towards active moving in the environment at this earlier age may well serve dispersal purposes, while becoming less *Actively Engaged* towards week 6 and 9 indicate periods of increased stasis. Higher levels of *Aggression* at this earlier age would also contribute to this dispersal, if individuals are acting aggressively towards one and driving each other away during encounters (Lorenz, 1963). Thus, higher scores along *Aggression* as well as *Active Engagement* dimensions may be indicators of a biological drive to disperse in the wild. Contributing to this is the assessment of the patterns by broods (Figs. 4, 5, 6), which indicate these same patterns of development, with the exception of I brood.

How might an increase in an individual's tendency from week 3 to week 6 to become *Aroused* or *Ready* contribute to the life history of these organisms? One possibility might be that this period from week 3 to week 6 marks a change in preference in prey type or size. Many species of cephalopods change diet as they grow larger (Hanlon & Messenger, 1996), but the early diet of *O. bimaculoides* in the wild has not been documented. Perhaps a strategy involving less *Aggression* but more highly *Aroused* or *Ready* behavioral tendencies is adaptive in dealing with different food items during this time. This may be the case if *Arousal/Readiness* tendencies mediate the distinction between preparation for fight or flight responses. Indeed, *Arousal* has been proposed to serve this purpose in mammals (Buss & Plomin, 1984), and choices surrounding such mechanisms surely must be adaptive in octopuses as well. This increase in the tendency to become more highly *Aroused or Ready* may be an indicator of the young octopuses moving from a more innate movement and attack feeding/interaction paradigm to one that includes a greater level of behavioral plasticity, mediated by increased *Arousal* input. The pattern of increased behavioral plasticity with age has been demonstrated in cuttlefish, which have an initial restriction of response to a particular pattern of visual stimulation that fades, rather than becoming more marked, with experience (Wells, 1962b; Messenger, 1973). The early period of weeks 3 to 6 in octopuses may mark a time of great change as well, with innately determined response patterns that give way to ones that are mediated by learning and experience. In octopuses, this development may be reflected as innately determined responses such as "attack" and "move" give way to those moderated by *Arousal* or choice mediated contexts.

An analysis of octopus development by brood also allows characterization of octopuses that may be different from the normative pattern for the entire group, as well as allowing generalizations to be made concerning a broad definition of heritability of temperament trait development. Higher variability can be seen in the pattern of development among broods of octopuses (Figs. 4, 5, 6) in comparison to the overall normative one (Fig. 3). Qualitatively, *Active Engagement* exhibits more variability among the three groups, especially among brood I members. I brood displayed a very different approach compared to the normative pattern on this dimension, with score levels on this dimension increasing through time for this group. Patterns of development at the brood level may represent the type of variability that is needed by these organisms in a highly fluctuating environment, like that of the near-shore shelf area. Presentation of variable patterns among brood types may be an adaptation of the species as a whole towards a highly complex and changing environment, and may represent a scenario where stabilizing selection is occurring in which genetic variability surrounding traits is conserved (Plomin, 1981). Brood analysis here also allows further characterization of the role of relatedness in the expression of temperamental qualities, but these results should be treated with caution, since no controls were able to be made concerning intra-brood interactions during the first 14 days of life (see Chapter 1). Interpretation of significant differences surrounding patterns of development here were still attributed to variance derived from the heritability of these traits, a conclusion also made by Herzog & Burghardt (1988) in their longitudinal study with garter snake litters.

Constancy and Change

The three approach analyses all contribute to the model of constancy and change in *Octopus*. In the first approach, an examination of the discrete behaviors, change is proportionately small, but is still seen both at the transition from week 3 to week 6 (Respiratory Change and Posture Change) as well as from week 6 to week 9 (Crawl). The significant changes in Respiratory Change and Posture Change may be paralleled from week 3 to week 6 by significant differences found along *Arousal/Readiness* and *Aggression* traits in the second approach analysis. An increase in Respiratory Change, which loads highly on *Arousal/Readiness* in the pattern matrix at week 3 (Table 1), would complement the increase seen in this dimension from this same time period. Similarly, a significant decrease in frequency of Posture Change would contribute to the significant increase in *Arousal/Readiness* scores found from week 3 to week 6 (negative loading for Posture Change on *Arousal/Readiness,* Table 1), while also contributing to the significant decrease in *Aggression* scores along this first time transition (a positive loading on *Aggression*, Table 1). The significant decrease in Crawl behaviors may also parallel decreased dispersal behaviors, and would indicate that dispersal was occurring at least throughout the first six weeks of life. This first approach documents overall stability of octopus behavioral development, but is useful when taken in context with the analysis of the broader dimensions, with the behaviors as indicators of their respective temperament dimensions.

In the second approach, which documented the pattern of development of broad behavioral dimensions for the entire group, consistency is seen along two of four dimensions across the first nine weeks of life (*Active Engagement*, *Avoidance/Disinterest*) while significant change is observed in two dimensions (*Arousal/Readiness*, *Aggression*) from week 3 to week 6 but not from week 6 to week 9. This indicates differential variability among different temperament traits, and also identifies the period of time from week 3 to week 6 in this organism as being a period of significant change when compared with week 6 to week 9.

The concepts of constancy and change are also illustrated in the brood level analysis, with consistent scores being observed along *Aggression* and *Avoidance/Disinterest* while change is observed along the *Active Engagement* and *Arousal/Readiness* profiles. The significant differences found in the patterns of development among broods can be found both at week 3 and week 9, but these differences are mainly reflected in the pattern of *Arousal/Readiness* scores.

Chapter Conclusions

Constancy and change is a developmental paradigm associated with studies concerning traits under the rubric of individual differences. Change may arise through the process of organism-environment interaction, which can be modeled as a dynamic self-regulating system (Thelen, 1990). In this model, change arises either within the individual or the developmental context, but most likely these realms are not strongly delineated, and the process of change arises through a process of self-assembly within these two parameters (Oyama, 1985). Continuity in individual differences may arise through heredity, stable situational characteristics, or consistent experience (Buss &

Plomin, 1984). Indeed, heritability (Buss & Plomin, 1984) and environment (Werner, 1989a,b; Thomas & Chess, 1986; Braungart, et al., 1992) are considered to be the major influences on the development of temperamental traits. Genetics may also contribute to the interpretation of constancy and change, since genes are thought to provide temperament with at least some degree of stability (Buss & Plomin, 1984), but are also known to be responsible for change as well (Plomin, 1986). Constancy and change has been well documented in studies concerning primates (Higley & Suomi, 1989; Stevenson-Hinde, et al., 1980a; 1980b) as well as other vertebrates (goats - Lyons, et al., 1988; fishes - Francis, 1990; wolves - Macdonald, 1983; pigs - Jensen, 1994; and gerbils - Cheal & Foley, 1985), but this is the first report of an invertebrate model of these developmental patterns in temperament.

Since animals were housed individually and kept under identical rearing conditions, the developmental results of variability among broods may be considered indicators of genetically determined responses. Caution is needed once again here, however, since there are confounding factors to the aspect of heritability in this study (Chapter 1). Heritability of behavioral response styles is a major aspect of the definition of temperament (Goldsmith, et al., 1987), as well as being of great interest to behaviorists (Alcock, 1993), and the results here as well as those found in Chapter 1 start to contribute to our knowledge of this relationship of behavioral expression among related individuals.

Almost nothing is known concerning the effects of environment upon the expression of temperamental traits in octopus, but in humans (Scott, Stewart, & DeGhett, 1974), as well as wolves (MacDonald, 1983), sensitive periods have been identified as time periods where external stimuli have the greatest long term impacts upon behavioral expression. The early life of *Octopus bimaculoides* could be characterized here as a transition time of greater variability among behaviors (weeks 3 through 6) followed by higher consistency in expression of temperament traits (weeks 6 through 9). Whether or not this higher variability from week 3 to week 6 in octopuses is one such "sensitive period", where external stimuli could make their greatest impacts, remains to be seen.

Finally, results indicate that changes in octopus early life history may be better documented at the level of temperament behavioral styles than observations of changes of discrete behaviors. Surely, interpretation of these broader dimensions allows wider interpretation of the life history of octopuses when compared to analysis of discrete behaviors alone. Researchers interested in the development of behavior in octopuses may want to consider these types of measurements in their studies and their interpretations as they continue to examine the early life of octopuses.

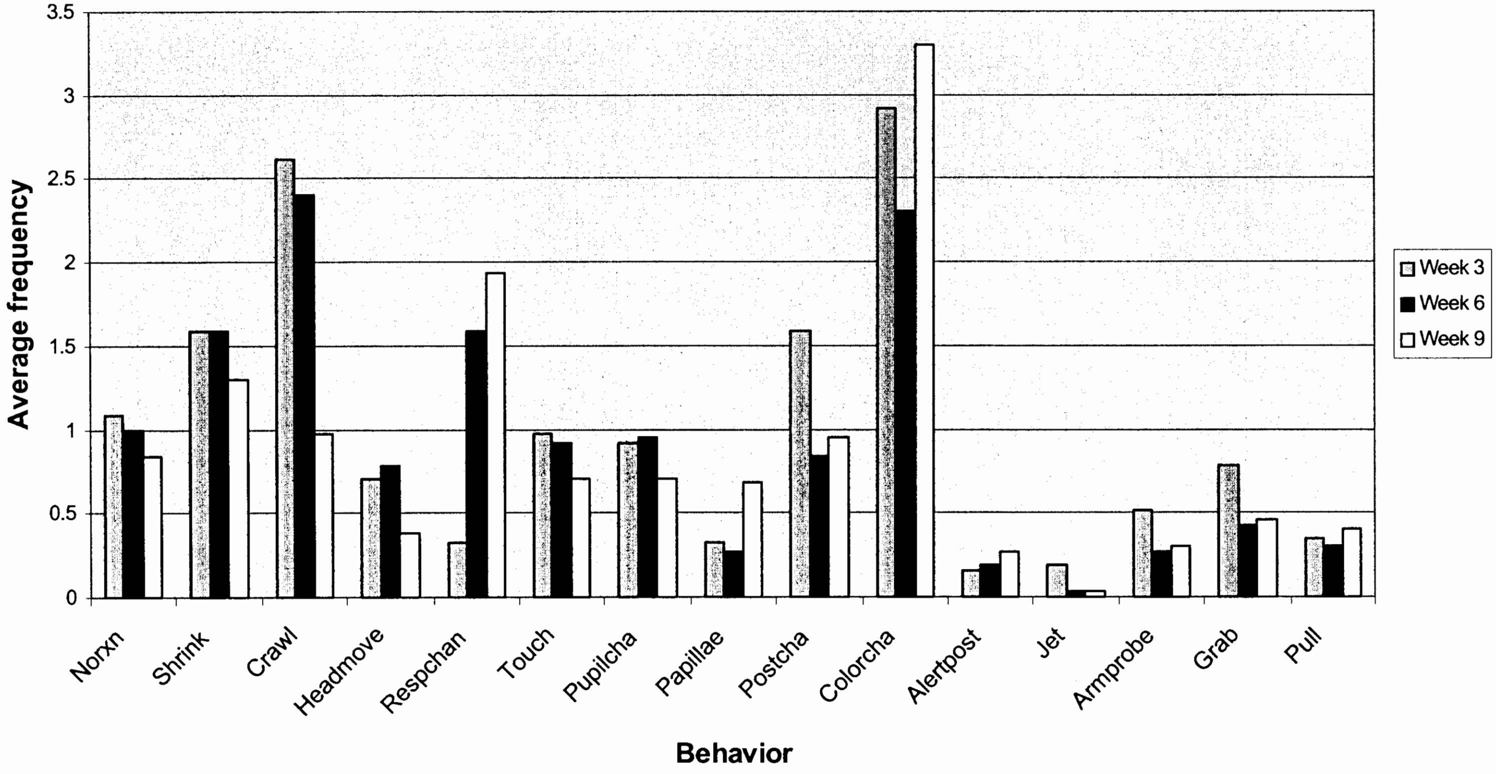


Fig. 2 - Mean Frequencies of Discrete Behaviors observed for *O. bimaculoides* at Week 3, Week 6, and Week 9 of life, n=37

Figure 3 - Profile of Compsosite Scores along Four Dimensions of Temperament at Week 3, Week 6, and Week 9 of life for *O. bimaculoides*, n=37

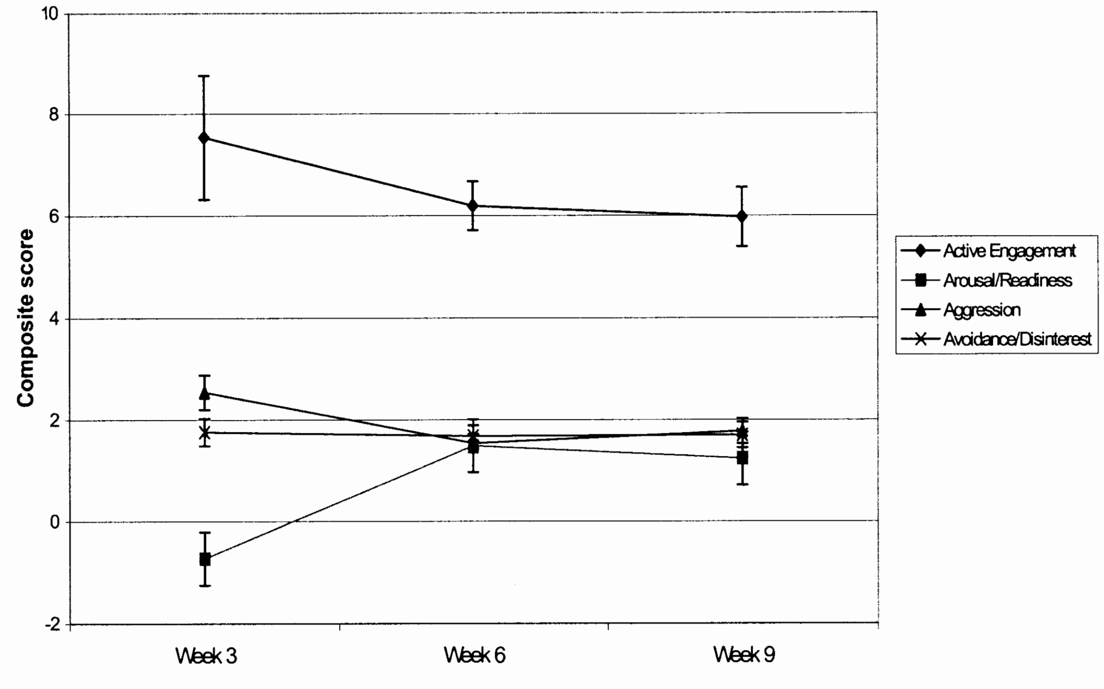


Figure 4 - Profile of Composite Scores along Four Dimensions of Temperament at Week 3, Week 6, and Week 9 of life, Y brood, n=19

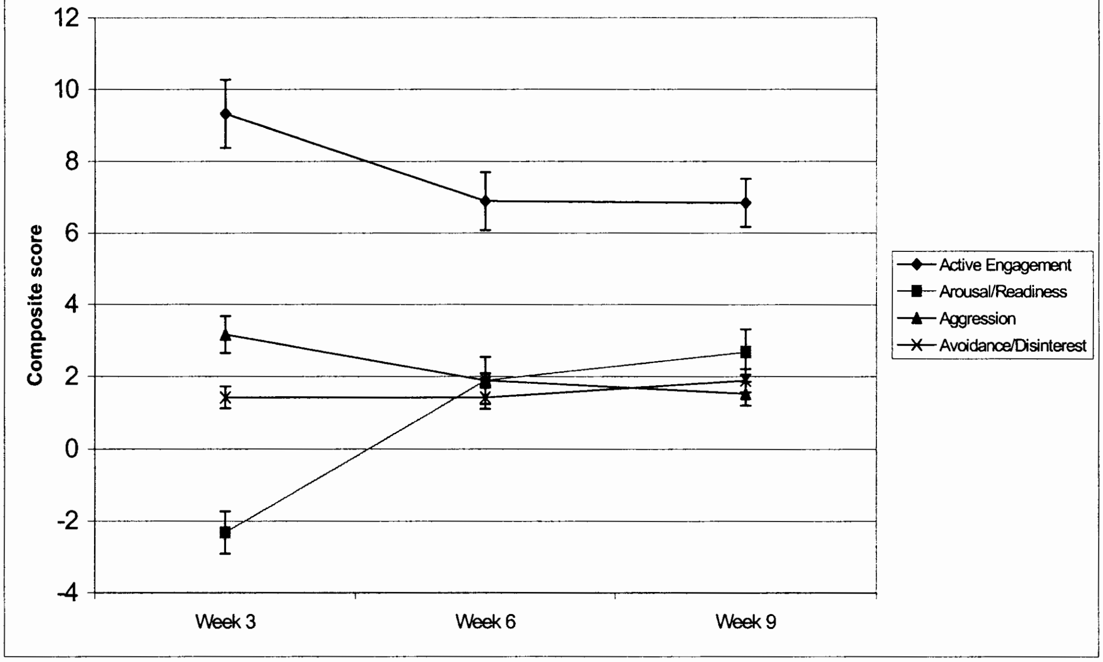
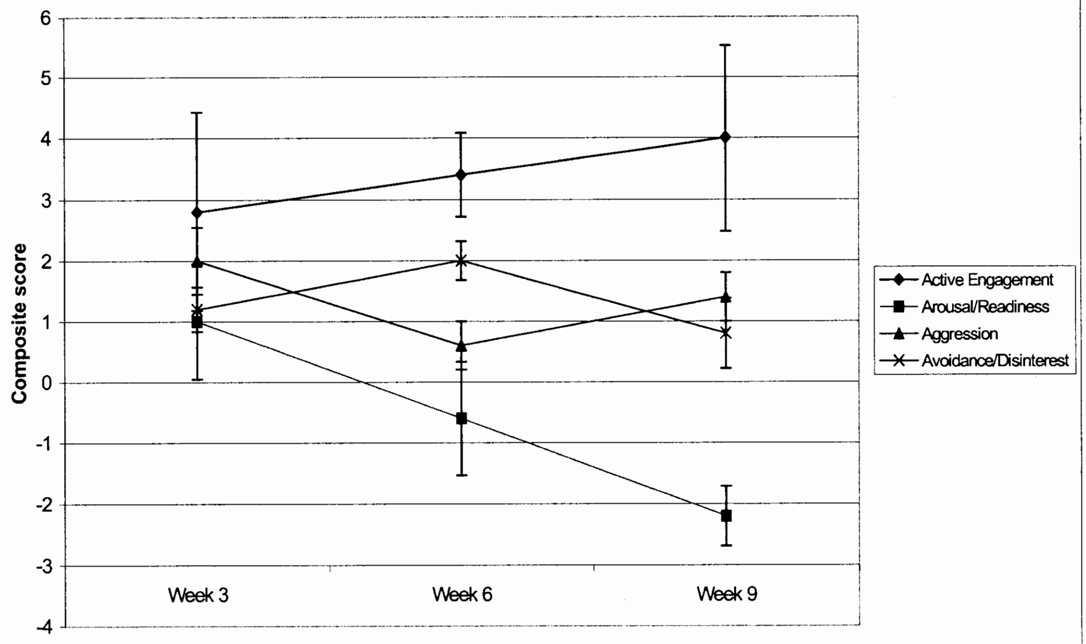


Figure 5 - Profile of Composite Scores along Four Dimensions of Temperament at Week 3, Week 6, and Week 9 of life, I brood, n=5



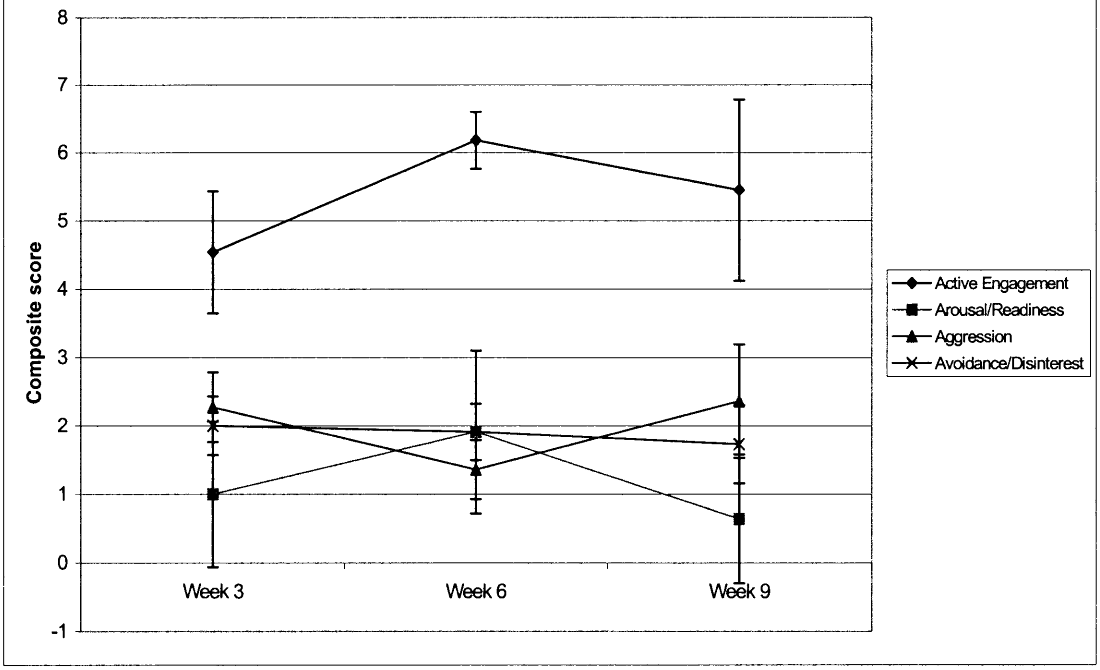


Figure 6 - Profile of Composite Scores along Four Dimensions of Temperament at Week 3, Week 6, and Week 9 of life, X brood, n=11

Final Conclusions

Temperament arises from a genetic endowment, is influenced by and influences the experience of the individual, and one of its outcomes is personality (Rothbart, et al., 2000). Temperament can be used to describe consistent individual differences in the behavior of organisms and are behavioral selective adaptations (Slater, 1981; Clarke & Boinski, 1995). By their third week life, *O. bimaculoides* display differences on at least four different temperament dimensions, described here as *Active Engagement*, *Arousal/Readiness*, *Aggression*, and *Avoidance/Disinterest*. Furthermore, the expression of these behavioral styles in octopuses is shown here to be influenced by the genetic background of the individual, both in their levels during week three of life and in their development in individual octopuses across the first nine weeks of life. The identification of these broad behavioral dimensions and their patterns across the early life provide us with part of the behavioral framework needed to begin to interpret the early life history strategies of *O. bimaculoides*.

Suggestions for further research

The present study illustrates how evaluation of the trait temperament in octopuses can inform us about proximal bases of intraspecific variation in behavior of this species. Further studies of variations along and among temperament dimensions in octopuses are needed in order to investigate other ecological specializations of behavior, including disparate diet and habitat requirements among different species, dominance hierarchies, foraging strategies, crypsis, and individual interaction with the environment. The role of underlying individual differences upon octopus behavior is in need of integration with these aspects of their life history, and a few topics seem particularly well suited for further study.

First and foremost, more species of octopus need to be tested for temperament traits, similar to the numbers of species that have been considered in primates (Torigoe, 1985). Because these types of tests are so new for these invertebrates, it is likely that studies to date (Mather & Anderson, 1993; the present study) have not yet measured the complete range of the psychology of these animals, especially when considering the diversity within this group (for example, deep water vs. shallow water octopuses). Within this species scenario, more detail in recording parameters is also needed, such as latency, duration, and direction of responses. Further studies with more species of octopuses combined with more detailed measurements will undoubtedly give us a wider range for understanding the development of behavior and how it may have evolved in this group of molluscs.

Secondly, examining how populations *within* species may be different would be particularly valuable, since inherited behavioural differences between populations can give information about the adaptive significance of behaviour in the various habitats occupied by a species (Olsen & Karlsson, 1991). For example, within primates, populations of squirrel monkeys have been shown to have higher between population differences in temperament dimensions than individual differences within either population (Martau, Caine, & Candland, 1985). An assessment of differing habitats among populations of the same species and its affects on each population's resulting individuality might further contribute to our ecological knowledge concerning octopuses. Octopuses that are observed across a wide geographical range, with no migration between genetically isolated populations would be particularly well suited. *Enteroctopus dofleini* may be an obvious candidate for this type of study, with its range along opposite shores of the Pacific and at least three subspecies within this distribution (Nesis, 1987; Hartwick & Barriga, 1997).

Thirdly, tests are needed to correlate behavioral responsivity to physiological reactivity in order to strengthen the links between genetic background and behavior in this group. To understand the proximate mechanisms that are the foundation of an individual's ability to behave, we need to understand the relation between an individual's genetic information and the development of the various systems (neural, hormonal, muscular) that make behavior possible (Alcock, 1993). Little is known about the physiological basis of behavior in octopus. Hormonal influences of behavior also are in need of study in this regard. It is worthwhile to note that from learning experiments it has been demonstrated that *Octopus vulgaris* has two almost independent learning mechanisms (Wells, 1962a; Young, 1983; Allen, Michals, & Young, 1986; Young, 1991), one concerned with learning to recognize situations by sight, while the other the learned recognition of things touched. Whether other structural systems in octopus parallel this design, such as the physiologic make-up of temperament, would seem a reasonable place to begin.

Finally, due to the possibilities of raising these animals in the laboratory (Hanlon, 1990), the effect of the developmental environment upon temperamental expression in octopuses is an excellent prospect for study. Environment is one of the major postulated influences on the development of temperament (Werner, 1989a,b; Thomas & Chess, 1986; Braungart et al., 1992), and these types of effects have been studied already in primates (Kraemer, 1992; Clarke, 1993), goats (Lyons, et al., 1988), and fishes (Katano, 1987). The octopuses would be an excellent invertebrate addition to our knowledge of the role of environment in the formation of individual differences.

Including individuality of octopuses in behavioral studies

We know now that octopuses have individual differences in behavior (Mather & Anderson, 1993; the present study), how can octopus researchers apply this knowledge to include the individual in their testing scenarios? Martin & Kraemer (1987) as well as Martin & Bateson (1993) have extensively considered the statistical effects of true individual differences. None of the information here presupposes what the reader can find in these two works, indeed, I have borrowed liberally from them. Due to the lack of the current use of their suggestions, at least in cephalopod research, I thought it important to include their work here along with my own personal suggestions in regards to the consideration of the individual octopus in behavioral studies.

True individual differences have two important statistical effects. First, by increasing the amount of variability within each group, individual differences make differences or correlations between experimental and control groups harder to detect since there is an increased amount of variability within each testing group, thus reducing the power of statistical tests. Secondly, individual differences make it more difficult to draw valid conclusions about individuals from the characteristics of groups, since statements about the group may be untrue for some or all of the individuals (Martin & Bateson, 1993).

Statistical methods used for analyzing behavioral data are normally based on inferences concerning populations rather than individuals (Martin & Kraemer, 1987), and there is a general need within the behavioral sciences to get away from thinking which assumes the generality of behavioral characteristics for a species and does not account for intraspecific variation in behavior (Slater, 1981; Martin & Kraemer, 1987).

Five broad statistical solutions have been proposed by Martin & Kraemer (1987) in order to assess the contributions of individuality in behavioral data. Each suggestion offers an option over taking single measurements from a group of individuals and lumping them together and making assumptions concerning the mean. These are summarized briefly here. After these suggestions I make a further proposal to those teuthologists concerned specifically with octopus behavior.

Taken from Martin & Kraemer (1987):

1) *Using a mean of repeated outcome scores for individuals* - obtaining repeated measurements of each individual's behavior after the experimental (or control) treatment has been applied and using a mean score from this individual in group measurements. This increases the reliability of the mean score for an individual, but may also reduce the feasibility of a study, and includes the possibility of introducing order effects (Martin & Bateson, 1986).

2) *Using a baseline score to obtain a measured index for each individual*- measuring

each individual's behavior before, as well as after, the treatment is applied. Each individual's response can then be expressed as an index of the change in its behavior due to the experimental or control treatment.

3) *Using a combination of repeated outcome scores and baseline measurements.*

4) *Using a longitudinal design* - measuring each individual's behavior repeatedly across time. From this data then a regression score can be generated for each individual and expressed as a coefficient. Coefficients, representing patterns of individual responses, can then be used for group analysis. Once again feasibility of these types of studies along with order effects are possible drawbacks.

5) *Increased sample sizes.*

Along with these I suggest we begin to consider the creation of a scalar method of rating or categorizing individual octopuses. By this, I mean a scale or reliable rating method that is based on discrete behaviors, whereby octopus researchers could perform brief observations to order individuals. Reliability of these measures would be a must of course, but these types of assessments could form the basis for maintaining separate groups of individuals for later statistical analysis. Particular dimensions of individuality, such as an *Activity* scale, might prove particularly useful in this regard. How might highly *Active* individuals score differently along a learning test than say, an extremely low *Actively Engaged* individual? And yet both strategies may be equally important to understanding the behavior of this genus. A rating method could group individual octopuses into either high or low *Activity* groups, and statistical analyses could confirm these ratings by the presence or absence of non-continuous lumps of scores, giving the researcher justification for treating groups of animals differently in interpretations. This standardized method of rating individuals in the laboratory would encourage researchers to further detail their findings, if assessments could be made quickly and accurately. Until then, the use of Martin & Kraemer's (1987) suggestions seems a reasonable starting point. If nothing else, comparison of individual plots of repeated measures upon the same tests to look for high individual variation between subjects can be performed, giving the researcher an idea of the influence of individuality upon their results (Slater, 1981; Martin & Bateson, 1986).

Final Remarks

Wells (1962a) commented that: "It is clear that knowledge about survival, e.g. hunting and avoidance, in adult cephalopods is not entirely innate, since individuals show such considerable differences and can so readily be trained to reverse their responses. Yet it is difficult to imagine how a young cephalopod could survive without some set of built-in instructions to guide its early experiments with its environment" (p. 38). The study of temperament provides the framework in which to study this dynamic between learning and innate behavioral responses in the early life history of octopuses.

By defining temperamental traits in *O. bimaculoides*, the *how* of octopus individuality is presented here along with possible hypotheses concerning the *why* of octopus temperament development. These types of studies contribute to our knowledge of the behaviors of octopuses and to our knowledge of the development of individual differences in a highly complex marine invertebrate. It seems apparent that with further study the octopuses will continue to provide us with an amazing array of behaviors that at first glance may seem so foreign to our ways of thinking, but through further examination will make perfect sense for the life of these soft-bodied marine molluscs.

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Appendix A - Operational Definitions of Behavioral Variables

Note: All behaviors could be displayed in any of the three test situations (Alert, Threat, or Feeding), unless otherwise noted; Means and Standard Deviations are given for week 3 frequency data only

No reaction: No visible reaction was noted. (mean (M)=1.22, standard deviation (SD)=1.00)

Shrink: A specific type of posture change: the arms and lower part of the body remained stationary while the head and mantle increased the distance from the stimulus. (M=1.55, SD=.85)

Crawl: Subject moves along the bottom or side of the testing compartment using the arms as its means of locomotion. (M=2.79, SD=2.97)

Head Movement: Subject makes vertical (up and down), horizontal (side to side), or vertical/horizontal (combination) movement of the head only. (M=.66, SD=.90)

Respiratory Change: Subject at least doubles its ventilation rate and then maintains this elevated rate for a 5-second period. (M=.53, SD=1.04)

Touch Stimulus: Subject initiates contact with the stimulus with one arm only (during Threat and Feeding tests). (M=.84, SD=1.07)

Pupillary Change: Subject's pupils are enlarged. (M=.89, SD=.89)

Papillae Change: Subject raises skin surface in papillae. (M=.25, SD=.79)

Posture Change: Head and body of subject changes position while maintaining a fixed point in space, this behavior was not directional in regards to the stimulus. (M=1.34, SD=1.52)

Color Change: Subject's overall skin color pattern is changed. (M= 2.38, SD=2.15)

Alert Posture: Subject raises eyes and the mantle is held at a 45 degree angle downward from vertical. The body is held fixed, and the arms are tucked close to the body and used to raise the head/eyes further in the vertical plane. (M=.18, SD=.42)

Jet or Swim: Subject moves by jet propulsion, with no contact to the bottom or sides of the container. (M=.37, SD=.89)

Arm Probe: Subject moves one or more arms laterally, maintaining contact with the container, but not touching any stimuli. (M=.59, SD=.88)

Grab Stimulus: Subject uses more than one arm contact a stimulus. (during Threat and Feeding tests). (M=.79, SD=.71)

Pull Stimulus: Subject, after Touching or Grabbing, maintains hold with suckers and attempts to shorten arm(s) (in Threat test only). (M=.26, SD=.53)

Appendix B - Naming of the factors

Active Engagement - The behaviors Crawl, Jet, Papillae Change, and Color Change were considered indicators of activity in this first dimension either through active moving (Crawl and Jet) or internal arousal (Papillae and Color Changing). *Activity* is a dimension that has been proposed for a number of different organisms across a wide range of taxa (Gosling & John, 1999), including humans (Buss & Plomin, 1984) and octopuses (Mather & Anderson, 1993). Young *O. bimaculoides* seem to differ from these other taxa (including *O. rubescens*) in the description of this dimension in that additional behaviors were interpreted as implying a broader meaning than simple activity would suggest. These additional behaviors are active arm movements (Arm Probe and Touch Stimulus), and suggest some sort of *Engagement* or active information gathering. *Octopus cyanea*'s approach to prey has been described as chemotactile exploration by Yarnall (1969), and Mather (1991) found extensive use of this same technique by juvenile *O. vulgaris* as well. Both used the term 'speculative hunting', which implies a type of information gathering, since these animals were probing beneath rocks and coral heads with their arms and suckers in order to 'feel' if there were any prey items. Mather & O'Dor (1991) also suggest that the learning tendency for octopuses dictates that they explore their habitat. *Active Engagement* encompasses these traits in young octopuses, and may be the initial substrate upon which these later behaviors evolve within the individual.

Arousal/Readiness - The second dimension was named to indicate behaviors that could be interpreted as an assessment exercise, or readiness towards a stimulus or object. Octopuses are known to use monocular parallax as a means of focusing on prey or other objects (Wells, 1962a; Maldonado, 1964; Hanlon & Messenger, 1996), a behavior that involves moving the eye physically between two points in order to triangulate a distance. The behavior of Head Move, which loaded highly on this dimension, involved moving the eyes in either a vertical up and down movement or a horizontal side to side, which could indicate possible parallax, or an attempt at focusing. Widening of the pupil in octopus when associated with movement of objects near the animal is also thought to reduce the depth of focus to permit more accurate ranging (Messenger, 1977; Budelmann & Young, 1993). Pupil Changes loaded highly on this dimension in combination with Head Movements, while a negative loading for Posture Change indicated that the animals had a tendency to not perform other body movements. *Active Engagement* movement behaviors did not load on this dimension as well. These three behaviors along with the lack of movement behaviors was interpreted as a type of focusing exercise, indicating *Readiness* towards an object, while not physically interacting with that object. Respiratory Changes are also included in this dimension and have been shown to be a measure of an octopuses motivational state (Boyle, 1983a; Chase & Wells, 1986), as well as being an indicator of arousal in cuttlefish (Boal & Ni, 1996). Respiratory change here was also interpreted as being an indicator of arousal in young octopus.

Aggression - The third dimension was characterized by behaviors that were performed directly and immediately towards the stimulus, either a brush or a crab. Grabbing stimulus, Pulling stimulus, and not Jetting from stimulus were all considered to be indicators of *Aggressiveness*.

Avoidance/Disinterest - Alert posture was interpreted as an individual's interest in a particular stimulus, and has been described as vigilant activity in the wild (Hanlon, Forsythe, & Joneschild, 1999). Alert Posture loaded negatively along this dimension with Papillae change and Shrinking, both of which loaded positively. Papillae change was interpreted as an indicator of arousal, and has been associated with alerting or arousal measures in *O. vulgaris* previously (Boyle, 1983a). Mather & Anderson (1993) also described *Avoidance* in adult *O. rubescens*, and it may be that this trait is important across a number of octopus species. It is also interesting to note that one of the fundamental axes of behavioral variation in a wide range of vertebrates is the *Shy-Bold* continuum (Wilson et al., 1993). This continuum can be described as *Shy* individuals reacting to unfamiliar situations by retreating or becoming quiet and vigilant, while *Bold* individuals act normally or actively approach stimulus in the same situations. Both *Avoidance* for *O. rubescens* as well as *Avoidance/Disinterest* for *O. bimaculoides* may be an invertebrate version of this *Shy-Bold* continuum.

It is interesting to note that behaviors that could be interpreted as indicators of arousal (Color changes, Papillae change, and Respiratory Change) loaded on three out of four dimensions in this study, suggesting that at least some sort of arousal contributes to these temperament dimensions. It may be that these behaviors indicate different types of arousal in octopuses, similar to separate arousal systems in humans (Buss & Plomin, 1984). No attempts were made here to distinguish between behavioral, autonomic or brain arousal here.

Appendix C - Culture Methods

In order to complete the study of individual differences in behavior in *O. bimaculoides* an inland, closed marine system was needed in which to hatch, raise, and maintain an adequate number of individual octopuses. Closed water marine cultures of octopuses have been reported previously in order to give researchers who are considering choice of subject organism information concerning maintenance requirements of this group of animals (Forsythe & Hanlon, 1980). Culturing of *O. bimaculoides* has also been performed by a number of researchers, most notably Forsythe & Hanlon (1988b) and Forsythe, DeRusha, & Hanlon (1984). Boyle (1983b; 1987) has reviewed our knowledge concerning the culturing of this genus, and his work remains an invaluable source of information to researchers interested in culturing these organisms for their own use.

For my purposes here a closed, 1900 l saltwater system was maintained at Portland State University from January 1997 through November 1999. Adult animals were housed intermittently in this system during 1/97 - 9/98, with housing and observations of young *O. bimaculoides* taking place between 9/98 - 9/99. The system was divided into two separate 660 l trays measuring 2.5 x .91 x .61 m and eight separate 76 l tubs measuring .91 x .33 x .33 m. Trays and tubs were used for holding animals while temperament testing took place in separate testing containers (see Chapter 1). Water levels were maintained at approximately 25 cm in all compartments. Artificial seawater was mixed in the laboratory using synthetic sea salts (Instant Ocean) and deionized water (DI). Supplemental nutrients were added once per week, and levels of ammonia, nitrates, nitrites, and pH were also tested weekly. Individual tubs and trays contained crushed oyster shell substrate and plastic sea grass beds along with shelter in the form of clay pots, small PVC tubing, and rocks. Temperature of the water was maintained at 18 degrees Celsius while salinities were maintained between 34-36 ppt. 190 l of water was replaced every 10-14 days to help maintain water quality when large numbers of young animals were present. Small amounts of DI water or seawater were added between water changes in order to maintain water levels and salinity. The system received overhead fluorescent lighting in addition to natural, indirect sunlight from large adjacent windows. The day/night light cycle of the fluorescent lights was kept approximately the same as the day length of Portland, OR during phases of experimentation.

Originally, six adult *O. bimaculoides* were obtained from Long Beach, CA (all animals collected by Chuck Winkler) and maintained in one large 660 l tray. Three months of preliminary observations were conducted with these animals. Data were collected on their movement patterns, interactions, mating behaviors, and other general behaviors. Matings were observed in the laboratory, and fertilized eggs were laid by two of the females present in this initial group; these young when hatched were used in temperament tests. Four additional females with eggs were also obtained from Long Beach, CA and the eggs were hatched. All six of these broods hatched within a month of one another, and the young were used for experimental testing until approximately 4.5 months of age, when deaths of all animals occurred over a period of one month due to unknown causes. Two more females with eggs were obtained from Long Beach, CA and these eggs were hatched four months after the first die off. This second set of young individuals was tested until approximately five months of age, when once again all animals died over a period of about a month due to unknown causes.

For feeding purposes, a variety of wild-caught organisms from the Oregon coast including littorinid snails (*Littorina* spp.), shore crabs (*Hemigrapsus* spp.), mysid shrimp (*Mysis* spp.), limpets (*Collisella* spp.), amphipods (*Corophium* spp.), and mole crabs (*Emerita* spp.) were used. Animals were occasionally fed captive raised freshwater mollies (*Poecilia* spp.) and live steamer clams (*Protothaca* spp.*)* purchased from a local grocery store. Littorinid snails and mysid shrimp were the major food source for the young animals, and were continuously available, except to animals that were being tested (see Chapter 1). Adults, when maintained, were fed three to four times a week, usually with shore crabs and steamer clams. Feedings were *ad libitum* during all phases of experimentation in order to avoid induction of an activity cycle based on feeding times (Wells, O'Dor, Mangold, & Wells, 1983).

Culture deaths of the young *O. bimaculoides*

The six adults originally obtained from the wild lived for six months in the system at which time each died of old age. All females with eggs that were obtained from the wild lived from two to four months after being placed in the system, at which time they also died, presumably of senescence. Although I was not able to rear an F2 generation, I was able to maintain adults, have them mate and reproduce, and then rear their young for five months.

The reasons that animals all died at five months of age are currently unknown, although hypotheses were generated concerning deaths. Since normal water quality measurements were continually recorded during all phases of experimentation, and since adults seemed to be unaffected, the existence of an age-specific bacterial or viral agent that was killing the young octopus is a real possibility. Forsythe, Hanlon, Bullis, & Noga (1991), Forsythe, Hanlon, & Lee (1987), and Hanlon, Forsythe, Cooper, DiNuzzo, Folse, & Kelly (1984) have begun to identify and examine the various pathogens affecting *O. bimaculoides* in the laboratory. Due to time limitations I was not able to identify any possible pathogens in this case. Another aspect of this pathogen scenario would be that a food organism from the Oregon coast may have introduced a disease organism, either not normally encountered by *O. bimaculoides*, or, not encountered in the high concentrations that may have been present in a closed water system. No attempts were made to sterilize wild caught food, and food was often collected during the summer months when the possibility of differing types of harmful blooms in the ocean may have been possible (Taylor & Horner, 1994; Horner, Garrison, & Plumley, 1997). A pathogen not normally encountered by these animals would have been devastating on the high concentration of octopuses present in the system. Alternatively, the immune system of the young may not have been as efficient in combating the possible pathogens present in the system as was that of the adults. Finally, due to the closed nature of the system, the possibility that an essential nutrient was not available for these animals during at this particular age is also a consideration. This would seem less likely, however, as frequent water changes combined with regularly added nutrient solutions were used.

During die off of animals, attempts were made to maintain and keep animals alive. A UV sterilization filter was installed into the water flow, with daily iodine treatments as well as daily antibiotic treatments (Streptomycin) for two weeks (for a review of antibiotic treatments in cephalopods, see Forsythe, et al., 1987). A fluidized bed filter was also installed during the third month of life during the second round of culture attempts. These attempts seemed to have little affect upon the death rate of individuals in the system. Specific pathogens have been identified in *O. bimaculoides* (Forsythe, et al, 1991; Forsythe, et al., 1987; Hanlon, et al., 1984), but animals here seemed to lack the identifying characteristics thus far known for pathogens, such as localized, visible lesions.

These types of culture issues may cause problems where a researcher is housing large numbers of animals in a closed-water system. It may be worthwhile for researchers who do not have access to open flow through systems to house animals in more than one system with separate water supplies. If one water supply became infected with a particular pathogen, having a separate facility would be a way to at least minimize the effects of the pathogen on all animals. Also, in case of electrical or mechanical break down, having separate systems would increase the chances of not losing all subject organisms. Ways to sterilize wild-caught or store-bought food may be helpful as well, and decrease the chances of introduction of disease organisms.

Although maintaining inland, closed cultures of *Octopus* spp. is challenging, it is worthwhile in that it allows a greater number of researchers to study this marine organism under semi-natural conditions. Many facets of our knowledge concerning this group are in need of study, and as more researchers attempt to culture their own subject organisms in inland laboratories our knowledge will certainly benefit.

Appendix D - First Observation of Behaviors

First occurrences of characteristic behaviors were recorded in young *Octopus bimaculoides* during the first six weeks of life post-hatching. Observations were made on seven different broods of young octopuses during daily routine maintenance checks of a culture system at Portland State (see Appendix C). Observations were not made during the addition of food items, although food was available during all times. Also, maintenance checks were made during random times of the day and did not follow any set schedule, however, most checks were made during daylight hours. For each listing the age of the animal is noted and attempts are made to integrate this knowledge with that currently found in the literature.

Cephalopods are known for rapid individual growth rates (Boyle, 1983b; 1987), and *O. bimaculoides* is no exception to this, with individuals capable of growing 3.56% of their body weight per day (Forsythe & Hanlon, 1988b). *O. bimaculoides* is a large-egged octopus, with benthonic young (Forsythe & Hanlon, 1988b) similar in appearance to the adults (Forsythe & Hanlon, 1988a). Hanlon & Messenger (1996) remark that it is obvious that many cephalopods must change their behavior considerably during their life cycle because of their great change in size, with physical ontogeny dictating changes in aspects of behavior such as feeding and movement patterns. Other changes can be seen during this physical ontogeny as well, such as new or different color patterns produced through the physical development of new elements, units and components of body patterns in the skin (Packard, 1982; 1985). It was my attempt here to observe the first occurrences of certain characteristic behaviors in *O.*

bimaculoides. Although by no means a systematic account, it does contribute to our species-specific knowledge concerning behavioral development in *O. bimaculoides*. Furthermore, even though examining broader dimensions of behavior such as temperament may be better indicators of change than examination of discrete behaviors (Chapter 2), it is still important to document the discrete changes that give rise to the underlying dimensions themselves.

Personal observations of the young and adults in observations at Portland State indicate that by ten weeks post-hatching, individual *O. bimaculoides* are able to perform most adult behaviors, with the exception of sexual or mating ones. It is my opinion that the behavioral capabilities of young *O. bimaculoides* increase exponentially across its early life. This fast behavioral development would allow young individuals to meet the requirements for survival in a complex environment, like that of the near-shore reef. This complex niche, along with the absence of physical protection against larger predators and their small size (animals in this study at 14 days post-hatching (n=73): mean mantle length = 6.5 mm; mean wet weight = 1.12 g) could contribute to this animal's heavy reliance upon learning, intelligence, and complex behavior (Mather, 1995).

This account will hopefully contribute to the overall scope of this thesis work by further detailing the developmental record of discrete behaviors in this species, a process begun in Chapter 2. Furthermore, this account may be of use to researchers who are interested in studying the young of this species, in that it gives them an approximate timetable in which to expect certain characteristic behaviors. Finally, it is given in hopes that the obvious missing pieces here will encourage other researchers to perform more systematic, detailed accounts of the behavioral development of octopuses. Further systematic observations of the young of this species as well as others will no doubt contribute greatly to our knowledge of young *Octopus* spp. and their life history requirements.

Ontogeny of Behaviors in Chronological Order

1a) *Immediately upon hatching*. Animals when disturbed visually perform the **Two Raised Arm** posture (Forsythe & Hanlon, 1988a). Here this posture is described as the animal holding up the first pair of arms with arm tips slightly turned downwards at the ends while all other arms are held down and underneath the body. If the animal is swimming the arms held beneath the body are curled at their ends as well. This behavior has been described previously for *O. bimaculoides* by Forsythe & Hanlon (*ibid.*) but was not noted in their individuals until the end of the first week of life, and then only while animals were walking. Packard & Sanders (1971) also describe this posture as a component of the Flamboyant display in *O. vulgaris*, however, the Two Raised Arm posture here in *O. bimaculoides* differs from this latter description since no papillae components were performed as described in Flamboyant. Among other coleoids, Moynihan & Rodaniche (1982) have documented this posture in the Caribbean reef squid, *Sepioteuthis sepioidea*, and various species of cuttlefishes also hold the first pair of arms above the head in various displays (Hanlon & Messenger, 1996).

b) *Immediately upon hatching.* Animals were observed swimming to the sides of the holding tank where they would attach themselves to the side, becoming all dark in color while curling the arms underneath the body and assuming a smooth contour outline along the body, arms, and attachment point to the side of the tank. The mantle was held at a 45-degree angle to the body, while held elongate, smooth, and pointed at the posterior apex. This behavior was performed among a large number of black, smooth, small littorinid snails that were climbing on the sides of the holding tank against a gray background. Although the color of the animals was black and not gray, this behavior was interpreted as **a crypsis or camouflage** behavior, since the young were identical in color, shape and size to the snails. Many cephalopods are well known to perform general background resemblance behaviors (Hanlon & Messenger, 1996), but no descriptions of this behavior at such an early age have been reported.

c) *Immediately upon hatching.* The **first interaction** between two individuals of Y brood within 15 minutes of hatching. Both individuals upon touching immediately jet from one another, possibly indicating both *Active Engagement* and *Avoidance/Disinterest* behaviors. Since this is the first reaction to another octopus immediately upon hatching, this type of response would appear to have at least some sort of innate component, and may contribute to the distribution of related young in the field.

2a) *Five days post-hatching.* When disturbed visually during counts of individuals, all animals are either in **Uniform Light Phase**, **Uniform Dark Phase**, or weak **Disruptive**

(Forsythe & Hanlon, 1988a). No blending of colors such as mottle patterns or combinations of lights and darks occurred in these animals during the first week of life, which confirms previous observations (Forsythe and Hanlon, *ibid.*).

3a) *2 weeks post-hatching.* First observation of **modification of the environment** by an individual. An octopus was observed sitting in a hole made in the substrate from digging with the funnel.

b) *2 weeks*. First **papillae** and **Acute Mottle** (Forsythe & Hanlon, 1988a) coloration patterns observed. Papillae are observed both over the eyes and on the mantle itself. Forsythe and Hanlon (*ibid.*) described acute Mottle in their animals at three weeks post-hatching.

c) *2 weeks*. First **frontal white spots** observed on front of body, just below eyes. These white spots have been described previously in a number of *Octopus* spp. (Packard & Sanders, 1969; Packard, 1988a; Hanlon, 1988; Anderson, 1995) and are thought to be used as disruptive or deflective marks (Packard, 1988a; Hanlon & Messenger, 1996).

4a) *2.5 weeks post-hatching.* **Cleaning behavior**, described as 'grooming' in Mather (1998) in her summary of this behavior across five species of octopuses and 'cleaning' in *O. vulgaris* by Packard & Sanders (1971). Here in *O. bimaculoides* it is described as an amorphous behavior, with the first 3 pairs of arms curled onto themselves and twisting as they are dragged across the front of the head/body towards the rear of the mantle, arms curling in two dimensions and moving in a third. The body is brown or dark in color, but adults are also observed performing this in a full mottle (pers. observations). The 4th pair of arms is not visible from the front of the animal.

b) *2.5 weeks*. **Full body passing cloud** performed. Forsythe and Hanlon (1988a) noted this behavior in *O. bimaculoides* at 5 weeks of age.

5a) *3 weeks post-hatching*. **Bilateral color pattern** observed, with left half of animal's body displaying full mottle (this was the side closest to a human finger moved towards the animal in the water), while the other half of entire body was a pale gray coloration. This has been termed a half-and-half coloration or unilateral effect in other cephalopods (Packard & Sanders, 1971) and is an indication of their bilateral brain control (Hanlon & Messenger, 1996).

b) *3 weeks*. First observation of a physical change in the external **appearance of the eyes**. Previous to this time the eyes could be described simply as black dots in appearance; now, pupils are visibly apparent and color changes in the immediate epidermis surrounding the eye are common. This is also the first occurrence of dark **Eye Bars** on both eyes. Eye bars consist of a dark stripe above and below the eye, running parallel to the long axis of the body, and are described in Forsythe and Hanlon (1988a) as occurring at week 3 of life.

6a) *4 weeks post-hatching*. **Yellow and orange** color components first begin to appear in the range of color patterns in the skin.

b) *4 weeks*. **Ocelli** become prominent in displays used by octopuses, including

ocelli flashing. Ocelli flashing consists of hues of blue flashing many times within a given second (approx. freq. 25 ms). The entire behavior lasts for 5-6 seconds, and was observed under conditions of a visual stimulus (researcher) standing over the tank. When not prominent the ocelli appear to match the background color of the skin or are visible but are of a brown hue. Ocelli become prominent in other color patterns at this age as well, especially Acute Mottle. Forsythe & Hanlon (1988a) note this behavior at 3 weeks of age. The purpose of the ocelli in color patterns is not entirely known, but is thought to be used for deflective markings, especially during deimatic displays (Hanlon & Messenger, 1996).

7a) *5 weeks post-hatching*. **'Facemask coloration'**. An animal in response to a visual stimulus (human face) responds with a mantle that is extended, with a posterior mantle tip, but the rest of the mantle is smooth. Older animals are observed to display this behavior as well, but with papillae as a textural component on the mantle. The background color of the mantle in this coloration is a light brown/green sheen (Forsythe & Hanlon, 1988a describe Chronic General Mottle, with a typical greenish hue, at 3 to 4 months of age), with a general mottle appearance. Dorsally along the length of the mantle there is a longitudinal black strip that starts behind the eyes and runs to the posterior apex of the mantle. Along its length there are three black bars that circumnavigate the mantle and intersect with this longitudinal stripe. This general color pattern has been described as Disruptive in squid and cuttlefishes (Moynihan & Rodaniche, 1982; Hanlon & Messenger, 1996), and Packard & Sanders (1971) also describe this color pattern in *O. vulgaris*.

8a) *6 weeks post-hatching*. **Unilateral Passing Cloud**, displayed in response to another octopus's approach. The approaching octopus was on the side of the body that the Passing Cloud was displayed. Forsythe & Hanlon (1988a) describe Passing Cloud as being displayed during interactions by 'aggressive' five-week-old *O. bimaculoides*. Both Passing Cloud and Unilateral Effect were described earlier in the animals here, yet this is the first occurrence observed of a combination of these patterns.

b) *6 weeks*. The **Response to threat** is performed in one of two ways. Both involve the animal turning all black and holding up the first pair of arms (Two Raised Arms) while increasing respiratory rate. In the first scenario the animal jets away while still holding this posture; in the other the animal holds onto the substrate where it was disturbed and performs a 'Jet Hold' response. 'Jet Hold' involves heavy jetting which moves the body of the animal up and down in the water column while the ventral rear arms remain attached to the substrate, holding the animal down. In motion the animal's entire body and arms bob up and down with the first pair of arms held in Two Raised Arms posture. This behavior is performed this behavior for 1-2 min. Later, 'Jet Hold' is observed in sexually mature individuals in the laboratory as well, but in adults is performed during Chronic General Mottle color components (pers. observations).

c) *6 weeks*. **Use of an object**. An individual uses a crab carapace from crab eaten earlier as a 'door', holding the carapace up in front of the body with the web while in its den with only the eyes exposed. This is a second sighting of modification of the environment.