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**Evaluation of meristic characters for  
determining hoki stock relationships**

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**Final Research Report for  
Ministry of Fisheries Research Project HOK1999/05  
Objective one**

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## Final Research Report

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  - 7. Executive Summary**

Eight meristic characters were evaluated as stock discrimination markers for hoki in the New Zealand EEZ. Five characters, the number of rays in the first dorsal fin, left pectoral, left pelvic fin, the number of gill rakers on the first gill arch, and the number of vertebrae were counted in 3 year old hoki collected from the two main spawning sites in Cook Strait and the west coast South Island in 2000. Only pelvic fin ray counts showed no variation within and between spawning sites. There was a significant difference in gill raker counts between sites, suggesting that the 3-year old fish were derived from two groups of fish exposed to different environmental conditions during the larval and/or early juvenile stages.

### 8. Objective

1. To determine stock structure and spawning fidelity of hoki (*Macruronus novaezelandiae*).

#### Specific Objective

1. To determine the feasibility of using meristic markers to differentiate between spawning stocks of hoki.

## 9. Methods

### 9.1 Background

Hoki support New Zealand's largest fishery with a TACC of 250,000 t. Although managed as a single stock under one TACC, hoki are assessed as two stocks, western and eastern with two major spawning areas off the west coast of the South Island and in Cook Strait (Annala *et al.* 2000). The current hypothesis, adopted by the stock assessment working group, is that juveniles from both spawning stocks mix on the Chatham Rise and recruit to their respective stocks as they approach sexual maturity. There are consistent differences in growth rate between fish on the two main spawning grounds (Horn & Sullivan 1996) which suggests that fish return to the same spawning ground each year, with limited mixing post recruitment. However it is not known if mature fish return to their natal spawning grounds. Most stock discrimination methods applied to hoki to date are either unsuitable (Horn & Sullivan 1996, Livingston & Schofield 1996), or have been insensitive (Smith *et al.* 1981, Kalish *et al.* 1996, Smith *et al.* 1996, D'Amato *et al.* 1999), to determining natal site fidelity.

Meristic characters, such as the number of vertebrae and numbers of fin rays, were among the first biological markers used to determine stock relationships of marine fishes (Heincke 1898). The methods were largely replaced by direct genetic methods developed in the 1970s. Meristic characters are determined early in the life cycle, for example the number of vertebrae is determined during the embryonic stages (Taning 1946, Fahy 1976 Lindsey 1988). The meristic characters have a genetic basis (Christiansen *et al.* 1988), but the number of vertebrae and fin rays can be modified by environmental factors, such as water temperature, so that population differences are due largely to environmental rather than genetic variation (Taning 1946, Fahy 1972, Brander 1978, Hulme 1995). Thus the meristic characters provide a tool that is determined early in the life cycle, and which has the potential to determine natal site fidelity.

Sample differences in meristic character require care in interpretation. Some meristic characters exhibit temporal and year class variation within fish stocks (Blouw *et al.* 1988). Furthermore, not all meristic characters have been useful for stock discrimination; for example Sharp *et al.* (1978) reported differences among samples of capelin *Mallotus villosus* with morphometric but not meristic characters. Therefore prior to undertaking large scale regional comparisons of hoki this pilot study was undertaken to determine the potential of meristic characters to discriminate between samples taken from the two main spawning areas.

### 9.2. Sample and data collection

Samples of whole hoki were collected from the two main spawning areas, Cook Strait and West Coast South Island, during the 2000 winter spawning season. One hundred undamaged 3 year-old fish (50 males and 50 females) were collected from each area. The west coast South Island (WCSD) specimens were collected aboard the RV *Tangaroa* during voyage TAN0007. The Cook Strait (COOK) specimens were collected from Sanford and Sealord commercial vessels landing in Nelson.

For both areas the catch was sorted to find small, undamaged specimens of the appropriate size range (COOK: 51–56 cm; WCSI: 53–58 cm). Specimens were checked for sex by slicing open the gut cavity to reveal the gonad, and 50 males and 50 females frozen whole from each area.

In the laboratory whole specimens were thawed and X-rayed using a Philips K140 Be portable X-ray unit in NIWA. The film was Kodak T-Mat-G/RA (35 x 43 cm) with settings 50kv, 10 sec, 2 ma. Films were developed in an automatic system at Wellington Hospital.

Vertebrae were counted from radiographs viewed on a light table. Fin rays and gill rakers were counted from the same thawed specimens. Counts of paired fin rays were made on the left side of each specimen. Counts of all the gill rakers on the first gill arch were made on the left gill arch of each specimen.

Initial fin ray and gill raker counts were made on ten specimens, five from each area, for the characters listed in Table 1. Three characters were not counted in further specimens. Firstly the number of lateral line scales could not be counted in the trawl caught samples as most specimens had lost their scales. The large number of rays in the second dorsal fin and anal fin were difficult to count in the thawed specimens. Repeat counts on the same specimens produced different counts, possibly due to a lack of a clear landmark between the second dorsal and anal fin.

The following counts were made on all thawed specimens: total rays in the first dorsal fin, left pectoral, left pelvic fin, and the total gill rakers on the first gill arch on the left side. The vertebrae were counted in the same specimens. The scientific literature was searched for data on hoki meristics.

### **9.3. Data analyses**

Differences in meristic characters between the COOK and WCSI samples were tested by multivariate analysis of variance (MANOVA), where the dependent variables were {1<sup>st</sup> dorsal, gill raker, vertebral number, and pectoral fin rays}, and the independent variables were area and fish sex. The Pillai-Bartlett trace test (Hand & Taylor 1987) was used to test the significance of the independent variables. Mean values of individual characters were compared between areas, and differences were tested using t-tests (assuming equal variances in the two areas). For characters showing significant differences between areas, the effect of fish sex was tested via a t-test within each area.

## **10. Results**

### **10.1 Hoki meristic data**

Meristic counts in the literature are summarised in Table 1, three were from text books on fish identification, and one from an FAO catalogue (Cohen *et al.* 1990). None of the references stated the location or number of specimens analysed. No vertebral counts were given.

Meristic count data for the COOK and WCSI spawning site samples are summarised in Table 2. One specimen was found with an unusually low number of vertebrae. The vertebrae were re-counted and the low count confirmed; the specimen had typical counts for the other characters. The number of fin rays in the left pelvic fins showed no variation (Table 2), but was one higher than counts reported by Ayling & Cox (1982) and May & Maxwell (1980) for an unknown number of specimens.

Table 1: Meristic counts on hoki *Macruronus novaezelandiae* in the scientific literature (- = no information)

Reference	Cohen <i>et al.</i> 1990	Ayling & Cox 1982	May & Maxwell 1980	Last <i>et al.</i> 1983
No of fish tested	-	-	-	-
First dorsal fin	1 spine; 10-12 rays	12	12	12 smooth rays
Second dorsal fin	96-102	96	96	96
Anal fin	89-95	89	89	89
Left pectoral fin	15-18	-	18-20	18-20
Left pelvic fin	8	7	7	-
Gill rakers:				
first left arch upper	6-7	-	7	-
Gill rakers:				
first left arch lower	21-24	-	-	-
Lateral line scales	182	-	-	-

Table 2: Summary of meristic counts in two spawning site samples of hoki

Area	No. fish	Gill raker	1 <sup>st</sup> dorsal	Pectoral	Pelvic	Vertebrae
WCSI	99	27-31	11-13	14-18	8	76-80 (68*)
COOK	103	27-34	11-13	14-18	8	75-81

\* One specimen with a low number of vertebrae.

Multivariate ANOVA indicated that area had a statistically significant effect on {1<sup>st</sup> dorsal, gill raker, vertebral number, and pectoral fin rays} ( $P < 0.0001$ ), allowing for the effect of sex (Table 3). Applying two sample t-tests (assuming equal variances), only gill rakers showed a significant area difference. A plot of the number of gill rakers observed in COOK and WCSI is given in Figure 1. Testing gill raker counts by sex, there was no significant difference for COOK (means 30.04 vs 30.04, t-test  $P = 0.99$ ). For WCSI, the sexes were significantly different (means of 29.00 vs. 29.45, t-test  $P = 0.013$ ). One possible explanation is that there were some COOK females in the WCSI sample, but this would need to be tested with temporal counts on 0-groups and subsequent year classes.

Table 3: Results of multivariate ANOVA on four meristic characters in two samples of 3-year old hoki from Cook Strait and the west coast South Island spawning grounds

	1 <sup>st</sup> dorsal		G.raker		Pectoral		Vertebrae	
	m	f	m	f	m	f	m	f
<b>COOK</b>								
Mean	12.3	12.1	30.0	30.0	16.6	16.4	78.0	77.8
Se	0.09	0.12	0.19	0.13	0.15	0.19	0.23	0.26
<b>WCSI</b>								
Mean	12.1	12.2	29.0	29.5	16.5	16.8	77.7	77.6
Se	0.11	0.10	0.12	0.13	0.18	0.12	0.21	0.30
<i>P</i> from t-test	0.19	0.41	<0.001	0.002	0.69	0.12	0.26	0.67

## 10.2 Stock hypotheses for hoki in the New Zealand EEZ

There are 3 potential stock models to be considered for hoki within the New Zealand EEZ. These stock models are:

- one unit stock with exchange of larvae and adults between regions;
- two, or more, stocks that are genetically isolated spawning stocks (i.e., natal site fidelity); and
- two, or more, stocks that share a common gene pool, but have post recruitment isolation of adults (i.e., post recruitment site fidelity).

Under the first single stock model no repeatable significant differences would be expected between regional samples with any stock discrimination method. There are consistent differences in growth rate between fish on the two main spawning grounds (Horn & Sullivan 1996) which suggests that fish return to the same spawning ground each year, with limited mixing post recruitment (i.e., model 3). However it is not known if mature fish return to their natal spawning grounds. Preliminary morphometric data, based on small samples of hoki heads from the two spawning grounds, also indicate that the single unit stock model is inappropriate (Livingston & Schofield 1996). Samples from non-spawning areas were different to the spawning area samples, although the Chatham Rise sample resembled the COOK sample more than the WCSI, and the Campbell Plateau was closer to the WCSI than the COOK sample (Livingston & Schofield 1996). The hoki working group have adopted a two stock model under which the COOK and WCSI are assessed as two stocks, but the level of site fidelity, natal or post recruitment, is unknown (Annala *et al.* 2000).

Under the second model of genetically isolated stocks, i.e., natal site fidelity, then genetic differentiation might occur between spawning sites. Initial genetic approaches employing allozymes (Smith *et al.* 1981) found no significant regional differentiation. More recent DNA data, based on mitochondrial DNA haplotypes (Smith *et al.* 1996), and microsatellite DNA (D'Amato *et al.* 1999, D'Amato unpublished observations), would support the lack of genetic differentiation between the two spawning populations. Lack of genetic differentiation is typical of many marine species with a potentially long pelagic juvenile stage and opportunity for gene flow, even when post recruits show little movement among areas (e.g., the armorhead, *Pseudopentaceros wheeleri* Martin *et al.* 1992; and spiny lobster, *Panulirus argus* Silberman *et al.* 1994). In shallow water teleosts a negative correlation has been reported between genetic differentiation and dispersal ability (Waples 1987), and a similar relationship

probably applies to deepwater species. Black and smooth oreo, with very long pelagic juvenile phases, show no genetic differentiation (Smith *et al.* 2000). In contrast orange roughy *Hoplostethus atlanticus*, with eggs that hatch near the bottom (Zeldis *et al.* 1994) and early juveniles that are assumed to be demersal, show significant genetic differentiation among spatially isolated spawning groups from the Chatham Rise, east coast South Island, and Puysegur (Smith *et al.* 1997). Hoki juveniles remain in the pelagic environment for several months (May & Blaber 1989) and adults from the two spawning sites within the New Zealand EEZ exhibit no genetic differentiation.

The genetic similarity among the hoki spawning sites could be due to present levels of gene flow, particularly juvenile movement, or to historical gene flow between stocks which are currently isolated but have not evolved genetic differences. The overall genetic data support, and do not allow rejection of, the null hypothesis of a single stock of hoki within the New Zealand EEZ.

An alternative approach to determining natal site fidelity is microchemistry of the otolith nucleus (Campana *et al.* 1997, Thresher 1999). Kalish *et al.* (1996) measured trace elements in 30 hoki from the WCSI and 30 from the COOK spawning areas, and separated the analyses into “juvenile” and “adult” otolith sections. Eight out of 23 elements showed concentrations above the detection limits, and differences were found between the juvenile and adult samples but none between juvenile samples from the two spawning areas. Advances in technology enable the analysis of discrete points within whole otoliths, and such methods could be applied to hoki otoliths. However the extensive studies of otolith microchemistry undertaken by Thresher and co-workers have raised major concerns about the application of this approach to stock discrimination of oceanic fishes. Many of the micro-elements used in chemical stock discrimination studies are sensitive to post mortem handling procedures (Proctor & Thresher 1998). Several of the specific elements (Na, K, and S) commonly used in stock discrimination studies, including hoki (Kalish *et al.* 1996), have been shown to be sensitive to post mortem handling procedures (Proctor & Thresher 1998, Thresher *et al.* 1999). Furthermore when differences are found between sites (in other species) they are in general small and have not been confirmed with repeat samples (Proctor & Thresher pers. comm.). Thresher (1999) concluded that the microchemistry technique is inappropriate for open ocean species because the oceanic environment is sufficiently homogeneous so that regional differences in otolith composition are very small. This general conclusion on oceanic species is supported by the microchemistry data of Kalish *et al.* (1996).

Otolith features may provide a measure of growth rate during the first year and a potential maker of natal site fidelity. NIWA is examining otoliths from hoki aged 3+ from the 1992 cohort, from the WCSI and COOK spawning sites, and the Chatham Rise & Southern Plateau (non-spawning areas) as part of the Ministry of Fisheries Project MOF1999/01J. Differences between WCSI-Southern Plateau and COOK-Chatham Rise juvenile otoliths may indicate stock differences, and natal ground fidelity. Data derived from this project will be presented in another report.

Meristic characters offer an alternative tool to test for natal site fidelity. Meristic characters are genetically determined but can be modified by the environmental conditions. Meristic characters differ when fish are exposed to different conditions,

such as temperature, during the egg and larval periods. In contrast to selectively neutral genetic markers, meristic characters measure differences that are determined at each spawning event, and offer a tool to measure short term differences, as opposed to evolutionary differences, among stocks. The observed differences in gill raker counts between 3-year old hoki from the COOK and WCSI spawning areas indicate that the two samples were taken from groups of fish exposed to different environmental conditions during the larval and/or early juvenile stages. In salmon the gill rakers become visible late in the larval period and the full complement of gill rakers may not be apparent until the juvenile stage (Beacham & Murray 1986).

These limited meristic data reject the single stock hypothesis, and indicate that 3-year old hoki (in 2000) in COOK and WCSI were derived from two groups of fish exposed to different environmental conditions during the larval and/or early juvenile stages, and imply a level of natal site fidelity. These meristic differences need to be tested with samples from additional year classes and from other areas.

## **11. Conclusions**

1. Hoki exhibit variation in several meristic characters and these provide potential markers to differentiate between spawning stocks of hoki.
2. There are significant differences in mean gill raker count between 3-year old hoki from the Cook Strait and west coast South Island spawning grounds.
3. Additional samples should be collected to test these preliminary differences:
  - 3-year old hoki from other fishing grounds, in particular the Southern Plateau and Chatham Rise, should be tested for meristic characters to determine stock relationships with the Cook Strait and west coast South Island grounds;
  - juvenile hoki should be compared from nursery areas off the west coast South Island, Puysegur, and on the Chatham Rise;
  - additional year classes should be compared from the two spawning areas to test for temporal stability of the observed meristic differences;
  - water temperature and other physical oceanographic data for Cook Strait and the west coast South Island spawning sites should be reviewed to identify environmental differences among egg and larval areas.

## **12. Publications**

None.

## **13. Data Storage**

Meristic data are stored on the H drive in file HOKmeristic.



## References

- Annala, J. H.; Sullivan, K. J.; O'Brien, C. J. (Comps.) 2000: Report from the Fishery Assessment Plenary, May 2000: stock assessments and yield estimates. Unpublished report held in NIWA library, Wellington. 495 p.
- Ayling, A.M.; Cox, G.J. 1982: Collins guide to the sea fishes of New Zealand. Collins, Auckland. 343 p.
- Beacham, T.D., Murray, C.B. 1986: The effect of spawning time and incubation temperature on meristic variation in chum salmon (*Oncorhynchus keta*). *Canadian Journal of Zoology* 64: 45–48.
- Brander, K. 1978: The relationship between vertebral number and water temperature in cod. *Journal du Conseil International pour l'Exploration de la Mer*. 38: 286–292.
- Blouw, D.M., Saxon, S.D., Chadwick, E.M.P. 1988: Temporal variation of meristic traits within an Atlantic salmon (*Salmo salar*) stock and implications for stock identification. *Canadian Journal of Fisheries & Aquatic Sciences* 45: 1330–1339.
- Campana, S.E. + 18 others. 1997: Comparison of accuracy, precision, and sensitivity in elemental assays of fish otoliths using the electron microprobe, proton-induced X-ray emission, and laser ablation inductively coupled plasma mass spectrometry. *Canadian Journal of Fisheries and Aquatic Sciences* 54: 2068–2079.
- Christiansen, F.B., Nielsen, V.H., Simonsen, V. 1988: Genetics of Zoarces populations XV. Genetic and morphological variation in Mariager Fjord. *Hereditas* 109: 99–112.
- Cohen, D.M., Inada, T., Iwamoto, T., Scialabba, N. 1990: FAO Species catalogue. Vol. 10. Gadiform fishes of the world. FAO Fisheries Synopsis 125, Vol. 10.
- D'Amato, M.E., Lunt, D.H., Carvalho, G.R. 1999: Microsatellite markers for the hake *Macruronus magellanicus* amplify other gadoid fish. *Molecular Ecology* 8: 1086–88
- Fahy, W.R.E. 1972: Influence of temperature change on number of vertebrae and caudal fin rays in *Fundulus majalis* (Walbaum). *Journal du Conseil International pour l'Exploration de la Mer*. 34: 217–231.
- Fahy, W.R.E. 1976: The morphological time of fixation of the total number of vertebrae in *Fundulus majalis* (Walbaum). *Journal du Conseil International pour l'Exploration de la Mer*. 36: 243–250.
- Hand, D. J., Taylor, C. C. 1987: Multivariate Analysis of Variance and Repeated Measures. Chapman and Hall, London.
- Heincke, D.F. 1898: Naturgeschichte des herrings. *Abh. Dtsch. Seefisch. Ver* 2: 128–233.
- Horn, P.L., Sullivan, K.J. 1996: Validated aging methodology using otoliths and growth parameters for hoki (*Macruronus novaezelandiae*) in New Zealand waters. *New Zealand Journal of Marine & Freshwater Research* 30: 161–174.
- Hulme, T.J. 1995: The use of vertebral counts to discriminate between North Sea herring stocks. *ICES Journal of Marine Science* 52: 775–779.
- Kalish, J.M., Livingston, M.E., Schofield, K.A. 1996: Trace elements in the otoliths of New Zealand blue grenadier (*Macruronus novaezelandiae*) as an aid to stock discrimination. *Marine & Freshwater Research* 47: 537–542.
- Last, P.R., Scott, E.O.G., Talbot, F.H. 1983: Fishes of Tasmania. Tasmanian Fisheries Development Authority, Hobart. 563 p.
- Lindsey, C.C. 1988: Factors controlling meristic variation In Fish physiology Vol. X1B. Eds Hoar, W.S., Randall, D.J. Academic Press, New York. pp.179–274.

- Livingston, M.E., Schofield, K.A. 1996: Stock discrimination of hoki (*Macruronus novaezelandiae*, *Merluccidae*) in New Zealand waters using morphometrics. *New Zealand Journal of Marine & Freshwater Research* 30: 197–208.
- Martin, A.P., Humphreys, R., Palumbi, S.R. 1992: Population genetic structure of the armorhead *Pseudopentaceros wheeleri*, in the North Pacific Ocean: application of the polymerase chain reaction to fisheries problems. *Canadian Journal Fisheries & Aquatic Sciences* 49: 2386–2391.
- May, J.L., Maxwell, J.G.H. 1980: Trawl fish from temperate waters of Australia. CSIRO, Hobart. 492 p.
- May, J.L. Blaber, S.J.M 1989: Benthic and pelagic fish biomass of the upper continental slope off eastern Tasmania. *Marine Biology* 101: 11–25.
- Sharp, J.C., Able, K.W., Leggett, W.C., Carscadden, J.E. 1978: Utility of meristic and morphometric characters for identification of capelin (*Mallotus villosus*) stocks in Canadian waters. *Journal Fisheries Research Board Canada* 35: 124–130.
- Silberman, J.D., Sarver, S.K., Walsh, P.J. 1994: Mitochondrial DNA variation and population structure in the spiny lobster *Panulirus argus*. *Marine Biology* 120: 601–608.
- Smith P.J., Patchell G.J., Benson P.G. 1981: Genetic tags in the New Zealand hoki *Macruronus novaezelandiae*. *Animal Blood Groups and Biochemical Genetics*. 12: 37–45.
- Smith P.J., McVeagh S.M., Ede A. 1996: Genetically isolated stocks of orange roughy (*Hoplostethus atlanticus*) but not hoki (*Macruronus novaezelandiae*) in the Tasman Sea and Southwest Pacific Ocean around New Zealand. *Marine Biology* 173: 783–793.
- Smith P.J., Benson P.G. & McVeagh S.M. 1997: A comparison of three genetic methods for stock discrimination of orange roughy, *Hoplostethus atlanticus*: allozymes, PCR amplified mitochondrial DNA and random amplified polymorphic DNA. *Fishery Bulletin* 94: 800–811.
- Smith P.J., Proctor, C., Robertson, S., McMillan, P., Bull, B., Diggles. B. 2000: Stock relationships of black oreo in New Zealand waters. Final Research Report for Ministry of Fisheries November 2000.
- Taning, A.V. 1946: Stage of determination of vertebrae in teleostean fishes. *Nature* 157: 594–595.
- Thresher, R.E. 1999: Elemental composition of otoliths as a stock delineator in fishes. In press.
- Thresher, R., Proctor, C., Evans, K., Mackinnon, C. 1999: Completion of an analysis of stock structure of orange roughy, based on otolith chemical composition. Fisheries Research and Development Corporation Project No. 1996/111.
- Waples, R.S. 1987: A multispecies approach to the analysis of gene flow in marine shore fishes. *Evolution* 41: 385–400.
- Zeldis, J.R., Grimes, P.J., Ingerson, J.K.V. 1994: Ascent rates vertical distribution, and a thermal history model of development of orange roughy *Hoplostethus atlanticus* eggs in the water column. *Fishery Bulletin* 93: 373–385.

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**Figure 1. Gill raker counts in 3-year old hoki from two spawning sites, COOK and WCSI.**

